USE OF AN ALDOSTERONE RECEPTOR ANTAGONIST TO IMPROVE COGNITIVE FUNCTION

BACKGROUND OF THE INVENTION

Cross-Reference to Related Application

This application claims priority from U.S. Provisional Application No. 60/228,738 filed August 28, 2000.

Description of Related Art

Cognitive and mood dysfunctions are a group of disorders characterized by either confusion, disorientation, memory disturbances, behavioral disorganization, depression, and disordered autonomic functioning (e.g. altered activity rhythms, sleep, and appetite). In many cases, definable neuropathological or metabolic disturbances underlie these conditions; in other cases, the etiological basis remains unknown. Of historic significance, treatment of cardiovascular disease with antihypertensive agents such as reserpine often caused depression, leading researchers to hypotheses of the role of the adrenergic system in mental illness.

Previous studies have indicated a relationship between aldosterone levels and mood (Birmingham MK, Barta A, Solyom L, Lehoux JG, Vecsei P. Correlations between mood scores, LH, adrenocortical steroids, and urine volumes in a patients with a history of postpartum depression and monthly psychotic episodes. *Endocrin Res* 1998;24:595-599).

Spironolactone, an aldosterone receptor antagonist, has been demonstrated to be an effective treatment for mood disturbances in premenstrual syndrome. In a double-blind, placebo-controlled cross over study, 35 women with PMS were given one tablet of 100 mg spironolactone or placebo daily from day 14 of the menstrual

cycle until the first day of the following menstruation. (Wang M et al.; Acta Obstet Gynecol Scand. 1995; 74:803-8) Two pretreatment cycles were observed for diagnosis in each woman, followed by 6 treatment cycles with spironolactone and placebo applied in either the first or second 3 months. The treatment with spironolactone was associated with an improvement in PMS symptoms compared to placebo as judged by significant decrease in negative mood symptom scores ($p < 0.001$) and somatic symptom scores ($p < 0.001$). Spironolactone significantly improved irritability, depression, feeling of swelling, breast tenderness and food craving in comparison to placebo. A lasting effect of spironolactone was observed in women started with spironolactone after cross over to placebo. Additionally, other investigators have demonstrated similar efficacy. (Hellberg D et al. *Int J Gynaecol Obstet*; 1991; 34:243-8.)

The RAAS system also appears to have a role in cognitive function. One such study that demonstrated this used the Dahl salt-sensitive (DS) rat, a genetic model of salt-induced hypertension. These rats were maintained normotensive on a low salt diet, and the effect of angiotensin-converting enzyme (ACE) inhibitor cilazapril or the angiotensin II type 1 receptor antagonist (E4177) at low, non-antihypertensive doses on passive avoidance was examined. (Hirawa N et al. *Hypertension* 1999;34:496-502). The cilazapril treatments dose-dependently improved memory function and was associated with significant increases in hippocampal CA1 cells and capillary densities in the CA1 regions. Similarly, E4177 slightly improved the memory dysfunction observed in the aged DS, restored slightly the cells in the hippocampal CA1 region, but the capillary densities were not influenced by the receptor antagonist . These results indicate that blockade of the RAAS system, downstream of ACE, improves memory dysfunction.

A hallmark of efficient cognitive processing is the ability to cope with environmental change. Conversely, enhanced reactivity (e.g. anxiety) to repeated exposure to novel stimuli is inversely correlated with positive cognitive function.

For example, there is an aged, impair Long-Evans rat which demonstrate reduced performance in the Morris water maze task, reduced exploratory behavior in a maze, lower milk consumption when sugar is added, and more reactive to novelty on the hot plate test. (Rowe WB, Spreekmeester E, Meaney MJ, Quirion R, Rochford J. Reactivity to novelty in cognitively-impaired and cognitively-unimpaired aged rats and young rats. *Neuroscience* 1998 Apr;83(3):669-80) Central cholinergic blockade with scopolamine produces profound cognitive impairments in human and animal subjects. In rats, the enhanced reactivity to novelty can be ameliorated by prior treatment of spironolactone. (Smythe JW, Murphy D, Timothy C, Gul GH, Costall B. Cognitive dysfunctions induced by scopolamine are reduced by systemic or intrahippocampal mineralocorticoid receptor blockade. *Pharmacol Biochem Behav* 1997 Apr;56(4):613-21).

Corticosterone binds to central mineralocorticoid receptors with high affinity and to glucocorticoid receptors with a tenfold lower affinity. Corticosteroid hormones are able to restore changes in neuronal membrane properties induced by current or neurotransmitters. Mineralocorticoid receptors mediate steroid actions that enhance cellular excitability, whereas activated glucocorticoid receptors can suppress temporarily raised neuronal activity. (Joels M et al. Trends Neurosci 1992; 15:25-30)

Oitzl et al. examined the mineralocorticoid receptor-mediated effect of corticosterone on the control of the behavioral response of male Wistar rats to spatial novelty, using a model wherein an object is placed in the center of an open field. (Oitzl MS et al. Eur J Neurosci 1994; 6:1072-9) Adrenalectomy increased the rats behavioral reactivity towards the object, a response blocked by the administration of corticosterone (50 micrograms/kg s.c.). Pretreatment of the rats with an intracerebroventricular injection of the selective mineralocorticoid receptor antagonist RU28318 prevented the corticosterone-induced increase in behavioral

reactivity, while the blockade of glucocorticoid receptors with the antagonist RU38486 (100 mg/microliters) was not effective.

Clinical studies of hypertensive humans on captopril (ACE inhibitor) have implicated a negative effect of RAAS on mental acuity and job satisfaction-morale. (Croog SH, Sudilovsky A, Levine S, Testa MA. Work performance, absenteeism and antihypertensive medications. *J Hypertens Suppl* 1987 Feb;5(1):S47-54). Withdrawal from the trial because of lethargy and fatigue was significantly greater among patients on methyldopa and propranolol than among those receiving captopril. Since hypertension was managed similarly between the groups, RAAS appears to modulate these parameters of cognitive function independent of this effect.

Insight into the mechanisms underlying the improved cognitive function through RAAS, or more specifically, ACE, blockade have come from comparative studies with a so-called nootopic drugs, piracetam. Mice were trained in a passive avoidance situation and then amnesia was induced by electro-shock. Pretreatment of these mice with ACE inhibitors or piracetam significantly improved retention performance. Retention-sparing effects of piractem was prevented by the aldosterone receptor antagonist, epoxy-mexrenone; epoxy-mexrenone had no effect on the ACE-inhibited mice. (Mondadori C. and Etienne P. Nootropic effects of ACE inhibitors in mice. (Psychopharmacology (1990) 100:301-307). These results indicate that that ACE inhibitors and piracetam memory-improving effects work through two distinct mechanisms.

The negative effects of RAAS on cognitive function may also have implications for Alzheimer disease. Indeed, one study found a significant increase of ACE in the caudate nucleus, the frontal cortex, the parahippocampal gyrus, and the medial hippocampus in Alzheimer patents. (Arregui A, Perry EK, Rossor M, Tomlinson BE. Angiotensin converting enzyme in Alzheimer's disease increased

activity in caudate nucleus and cortical areas. *J Neurochem* 1982 May;38(5):1490-2).

Heart failure (HF) is a common clinical syndrome with considerable impact on health-related quality of life (HRQOL) and functional status. (Leidy NK, Rentz AM, Zyczynski TM. Evaluating health-related quality of life outcomes in patients with congestive heart failure: A review of recent randomized controlled trials. *PharmacoEconomics* 1999;15:19-46). The primary goals of treatment are the prevention of disease progression and amelioration of symptoms. Assessment of improvements in patients' symptoms and functional status requires self-reported measures of health status and HRQOL. Clinical practice guidelines for health failure suggest that HRQOL measures be used to assess the effectiveness of medical therapies. (Konstam M, Dracup K, Baker D, et al., Evaluation and Care of Patients with Left Ventricuylar Dysfunction. *AHCPR Publication No 94-0612*1994).

Depression is an important risk factor for mortality. Recognition of depressive and anxiety disorders in adolescents reduces morbidity, mortality, and lifetime risk for psychiatric illness and maladaptive behaviors. (Reeve A. *Med Clin North Am* 2000; 84:891-905.) Depression is a common clinical syndrome in the elderly, often resulting in attempted and/or successful suicide. A one-year study by Royner et al. examined 454 new patient admissions to eight Baltimore area nursing home facilities. (*Am J Med* 1993; 94:19S-22S) Major depressive disorder occurred in 12.6% of patients; an additional 18.1% had depressive symptoms. Most cases of depression were unrecognized and therefore untreated by nursing home physicians. Major depressive disorders were found to be an independent risk factor for mortality that increased the likelihood of death by 59% in the first year after diagnosis.

Schulz R et al. examined a total of 5201 men and women aged 65 years and older from 4 US communities participated in the study. (*Arch Intern Med.* 2000;

160:1761-8) High baseline depressive symptoms were associated with a higher mortality rate (23.9%) than low baseline depression scores (17.7%). Depression was also an independent predictor of mortality when controlling for sociodemographic factors, prevalent clinical disease, subclinical disease indicators, or biological or behavioral risk factors. When the best predictors from all 4 classes of variables were included as covariates, high depressive symptoms remained an independent predictor of mortality .

Another study by Herrmann et al. demonstrated that depressed mood is an independent risk factor for all-cause mortality in medical inpatients. (*Psychosom Med* 1998; 60:570-7) This study also demonstrated that identifying patients at risk does not require formal psychiatric diagnoses, but can be achieved by means of a short, routinely administered self-rating questionnaire.

Similarly, Ganzini examined patients in an academically-affiliated Veterans Affairs Medical Center. (*J Am Geriatr Soc* 1997; 45:307-12) In a thirty-month follow- up of an inception, one hundred veterans (half of whom had a depressive disorder), were recruited from inpatient medical and surgical units in 1990-1991. At initial evaluation all participants were older than age 65, cognitively intact, and medically but not terminally ill. At 30 months, 36 of the 100 subjects had died. Only two factors predicted mortality: severity of medical illness and depression. Accordingly, major depression in medically-ill older hospitalized veterans continues to be a risk factor for death 30 months after diagnosis.

Depression is also a significant sequela of stroke which contributes to increased morbidity and mortality in stroke survivors. (Bush. *Brain Inj.* 1999 13:131-7)

Summary of the Invention

The present invention comprises methods for preventing or treating cognitive dysfunction in a subject. The methods comprise administering an aldosterone antagonist to a subject susceptible to or suffering from cognitive dysfunction, wherein the aldosterone antagonist is administered in a quantity that is therapeutically effective in improving cognitive function or preventing progression of cognitive dysfunction.

Brief Description of the Drawings

Fig. 1-1 shows change in plasma renin activity and serum aldosterone with eplerenone dose.

Fig 1-2 shows change in systolic blood pressure with eplerenone dose.

Fig 1-3 shows change in diasytolic blood pressure with eplerenone dose.

Fig. 1-A shows X-ray powder diffraction patterns of Form H eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form L eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Fig. 2-A shows a differential scanning calorimetry (DSC) thermogram of non-milled Form L directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form L prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form L prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form L, and milling the

resulting Form L.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form H prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form H eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form L eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of eplerenone.

Fig. 3-D shows the infrared spectra (diffuse reflectance, DRIFTS) of eplerenone in chloroform solution.

Fig. 4 shows ^{13}C NMR spectra for Form H of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form L of eplerenone.

Fig. 6-A shows the thermogravimetry analysis profile for the methyl ethyl ketone solvate.

Fig. 7 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

Fig. 8 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 9 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 10 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 11 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 12 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table 7A.

Fig. 16 shows a half normal plot prepared using the cube plot of Fig. 15 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 17 is an interaction graph based on the results reported in Table 7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 18 shows a cube plot of Form H weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table

7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table 7A showing the interaction between starting material purity and endpoint temperature on final material purity.

Fig. 21 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 22 shows a DSC thermogram of amorphous eplerenone.

Detailed Description of the Preferred Embodiments

It has been discovered that administration of one or more aldosterone receptor antagonists in accordance with the present invention is effective in the treatment of a subject with one or more cognitive dysfunctions. Such cognitive dysfunctions which can be effectively treated in accordance with the present invention include, but are not limited to, psychoses, cognitive disorders, mood disorders, anxiety disorders, and disorders of personality. In one embodiment, the subject is a mammalian subject. In another embodiment, the subject is a human subject.

Subjects who can benefit from treatment according to the present invention include subjects who are affected by psychoses. Such psychoses include, but are not limited to, conditions where there is impairment of behavior and an inability to think coherently, to comprehend reality, or to gain insight into the presence of these abnormalities. Psychoses may or may not include symptoms of false belief and abnormal sensations.

Subjects who can benefit from treatment according to the present invention include subjects who are affected by cognitive disorders. Such cognitive disorders include conditions characterized by one or more of the symptoms of confusion, disorientation, memory disturbances, or behavioral disorganization.

Subjects who can benefit from treatment according to the present invention include subjects who are affected by mood disorders. Such mood disorders include conditions characterized by one or more of the symptoms of depression, bipolar disorders, persistent abnormalities of mood, and disordered autonomic functioning such as altered activity rhythms, sleep, and appetite.

Subjects who can benefit from treatment according to the present invention include subjects who are affected by anxiety disorders. Such anxiety disorders include conditions characterized by one or more of the symptoms of anxiety, panic, dysphoria, obsessions, irrational fear, rituals or compulsions, or disorders of patterns of behavior. Such disorders of patterns of behavior include abuse of alcohol or other substances, deviant eating patterns, hypochondriasis, and antisocial behaviors.

It has also been discovered that administration of aldosterone receptor antagonists according to the present invention is effective in improving cognitive function in individuals lacking remarkable cognitive dysfunction. Such improvement includes, but is not limited, short- and long-term memory, sleep patterns, reactivity to environment, fear acclimation, and anxiety acclimation.

Subjects who can benefit from treatment according to the present invention include subjects who are affected by one or more pathological conditions affecting the heart, kidney, and vasculature, wherein addition of one or more aldosterone receptor antagonists in accordance with the present invention to standard treatment is effective in the treatment of a subject with one or more cognitive dysfunctions.

Dosing

A Phase II dose-ranging study demonstrated that eplerenone was safe and effective in patients with mild-to-moderate hypertension. This parallel design, eight-week, double-blind, placebo-controlled trial randomized 417 patients to one of three total daily doses of eplerenone (50, 100, or 400mg) administered once daily or in a divided dose regimen, spironolactone 50mg BID, or placebo.

The adjusted mean changes (in mmHg) in diastolic blood pressure (DBP) and systolic blood pressure (SBP) from baseline to final visit are shown in Table 1A.

Table 1A

	Eplerenone						Spiro	Placebo
	25 mg BID	50 mg QD	50 mg BID	100 mg QD	200 mg BID	400 mg QD	50 mg BID	
Cuff DBP	-4.4	-4.5	-7.8	-4.4	-8.9	-8.7	-9.5	-1.1
Cuff SBP	-8.1	-4.4	-11.7	-7.9	-14.8	-15.0	-16.7	1.6
ABPM DBP	-4.1	-5.1	-6.6	-5.6	-9.0	-7.7	-8.7	0.4
ABPM SBP	-7.5	-6.2	-11.6	-9.6	-16.1	-13.7	-15.8	0.0

ABPM = Ambulatory Blood Pressure Monitoring QD = Once daily

Spiro = Spironolactone BID = Twice daily

Eplerenone was well tolerated, and the incidence of adverse events, including gynecomastia, was similar to placebo. There was an increased frequency of transient hyperkalemia, (i.e., ≥5.4 mmol/L) noted in the eplerenone 200mg BID and 400mg QD treatment groups. These elevated potassium levels were not associated with any cardiac related adverse events, and all resolved spontaneously

without intervention. Due to the surprising lack of serious hyperkalemia even at daily doses as high as 400 mg, the range under which epoxy-steroidal compounds may be administered according to the present invention is very broad.

Thus, subject treated according to the present invention will be initially dosed using an amount of aldosterone receptor antagonist in the range of 0.25 mg to 100 mg per day; preferably in the range of 5 mg to 50 mg per day.

The initial evaluation period (i.e. the period during which a subject receives an aldosterone receptor antagonist at an initial daily dose) will be from about one to four weeks of duration; preferably from one to two weeks.

After the initial evaluation period, blood and urine samples will be obtained for routine evaluation (i.e., commonly known as blood and urine chemistries). Additionally, cognitive dysfunction will be evaluated. If there are no contraindications to a dose increase (e.g. hyperkalemia), the daily dose of an aldosterone receptor antagonist will be increased by an increment from 10 to 100 mg per day, preferably from 20 to 50 mg per day.

In one embodiment of the present invention, dose of an aldosterone receptor antagonist will be increased in stepwise manner until a dose of 400 mg per day is attained or until hyperkalemia is detected or other contraindications observed.

Appropriate dosing can also be determined by monotoring plasma renin activity. As shown in Figure 1-1, increasing doses of eplerenone results in increased levels of plasma renin activity. Accordingly, subjects can be treated with doses of one or more aldosterone receptor antagonists according to the present invention by increasing doses of such antagonists in a step-wise manner until a maximal levels of plasma renin activity is achieved while, at the same time, maintaining serum levels of potassium within the normal range.

Appropriate dosing can also be determined by monitoring serum aldosterone levels. As shown in Figure 1-1, increasing doses of eplerenone results in increased

levels of serum aldosterone. Accordingly, subjects can be treated with doses of one or more aldosterone receptor antagonists according to the present invention by increasing doses of such antagonists in a step-wise manner until a maximal levels of serum aldosterone is achieved while, at the same time, also maintaining serum levels of potassium within the normal range.

Appropriate dosing can also be determined by monitoring systolic blood pressure. As shown in Figure 1-2, increasing doses of eplerenone results in decreased systolic blood pressure. Accordingly, subjects can be treated with doses of one or more aldosterone receptor antagonists according to the present invention by increasing doses of such antagonists in a step-wise manner until reduced levels of systolic blood pressure are achieved while, at the same time, also maintaining serum levels of potassium within the normal range.

Appropriate dosing can also be determined by monitoring diastolic blood pressure. As shown in Figure 1-3, increasing doses of eplerenone results in decreased diastolic blood pressure. Accordingly, subjects can be treated with doses of one or more aldosterone receptor antagonists according to the present invention by increasing doses of such antagonists in a step-wise manner until reduced levels of diastolic blood pressure are achieved while, at the same time, also maintaining serum levels of potassium within the normal range.

Biological Evaluation

Compounds

The term "aldosterone antagonist" denotes a compound capable of binding to an aldosterone receptor, as a competitive inhibitor of the action of aldosterone itself at the receptor site, so as to modulate the receptor-mediated activity of aldosterone.

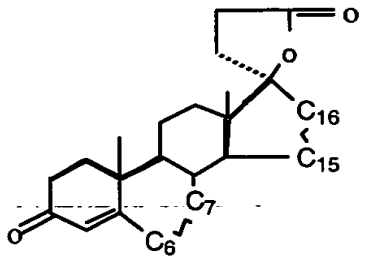
Many aldosterone receptor blocking drugs are known. For example, spironolactone is a drug which acts at the mineralocorticoid receptor level by competitively inhibiting aldosterone binding. This steroidal compound has been used for blocking aldosterone-dependent sodium transport in the distal tubule of the kidney in order to reduce edema and to treat essential hypertension and primary hyperaldosteronism [F. Mantero et al, Clin. Sci. Mol. Med., 45 (Suppl 1), 219s-224s (1973)]. Spironolactone is also used commonly in the treatment of other hyperaldosterone-related diseases such as liver cirrhosis and congestive heart failure [F.J. Saunders et al, Aldactone; Spironolactone: A Comprehensive Review, Searle, New York (1978)]. Progressively-increasing doses of spironolactone from 1 mg to 400 mg per day [i.e., 1 mg/day, 5 mg/day, 20 mg/day] were administered to a spironolactone-intolerant patient to treat cirrhosis-related ascites [P.A. Greenberger et al, N. Eng. Reg. Allergy Proc., 7(4), 343-345 (Jul-Aug, 1986)]. It has been recognized that development of myocardial fibrosis is sensitive to circulating levels of both Angiotensin II and aldosterone, and that the aldosterone antagonist spironolactone prevents myocardial fibrosis in animal models, thereby linking aldosterone to excessive collagen deposition [D. Klug et al, Am. J. Cardiol., 71 (3), 46A-54A (1993)]. Spironolactone has been shown to prevent fibrosis in animal models irrespective of the development of left ventricular hypertrophy and the presence of hypertension [C.G. Brilla et al, J. Mol. Cell. Cardiol., 25(5), 563-575 (1993)]. Spironolactone at a dosage ranging from 25 mg to 100 mg daily is used to treat diuretic-induced hypokalemia, when orally-administered potassium supplements or other potassium-sparing regimens are considered inappropriate [Physicians' Desk Reference, 46th Edn., p. 2153, Medical Economics Company Inc., Montvale, N.J. (1992)].

Another series of steroidal-type aldosterone receptor antagonists is exemplified by epoxy-containing spironolactone derivatives ("epoxy-steroidal compounds"). For example, U.S. Patent No. 4,559,332 issued to Grob et al

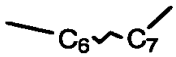
describes 9a,11a-epoxy-containing spironolactone derivatives as aldosterone antagonists useful as diuretics. These 9a,11a-epoxy steroids have been evaluated for endocrine effects in comparison to spironolactone [M. de Gasparo et al, J. Pharm. Exp. Ther., 240(2), 650-656 (1987)].

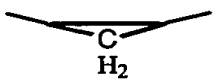
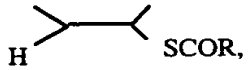
Derivatives are intended to encompass any compounds which are structurally related to the aldosterone antagonists or which possess the substantially equivalent biologic activity. By way of example, such inhibitors may include, but are not limited to, prodrugs thereof.

Non-epoxy-steroidal aldosterone antagonists suitable for use in the present methods include a family of spirolactone-type compounds defined by Formula I:

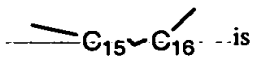


(I)

wherein  is

 or  SCOR,

wherein R is lower alkyl of up to 5 carbon atoms, and

wherein  is

Lower alkyl residues include branched and unbranched groups, preferably methyl, ethyl and n-propyl.

Specific compounds of interest within Formula I are the following:

7α-acetylthio-3-oxo-4,15-androstadiene-[17(β-1')-spiro-5']perhydrofuran-2'-one;

3-oxo-7α-propionylthio-4,15-androstadiene-[17((β-1')-spiro-5']perhydrofuran-2'-one;

6β,7β-methylene-3-oxo4,15-androstadiene-[17((β-1')-spiro-5']perhydrofuran-2'-one;

15α,16α-methylene-3-oxo-4,7α-propionylthio-4-androstene[17(β-1')-spiro-5']perhydrofuran-2'-one;

6β,7β,15α,16α-dimethylene-3-oxo-4-androstene[17(β-1')-spiro-5']-perhydrofuran-2'-one;

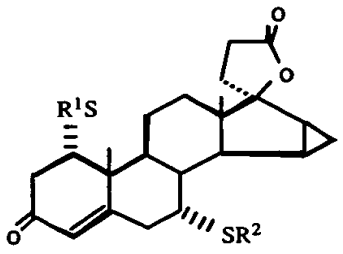
7α-acetylthio-15β,16β-Methylene-3-oxo-4-androstene-[17(β-1')-spiro-5']perhydrofuran-2'-one;

15β,16β-methylene-3-oxo-7β-propionylthio-4-androstene-[17(β-1')-spiro-5']perhydrofuran-2'-one; and

6β,7β,15β,16β-dimethylene-3-oxo-4-androstene-[17(β-1')-spiro-5']perhydrofuran-2'-one.

Methods to make compounds of Formula I are described in U.S. Patent No. 4,129,564 to Wiechart et al. issued on 12 December 1978.

Another family of non-epoxy-steroidal compounds of interest is defined by Formula II:



(II)

wherein R^1 is C_{1-3}-alkyl or C_{1-3} acyl and R^2 is H or C_{1-3}-alkyl.

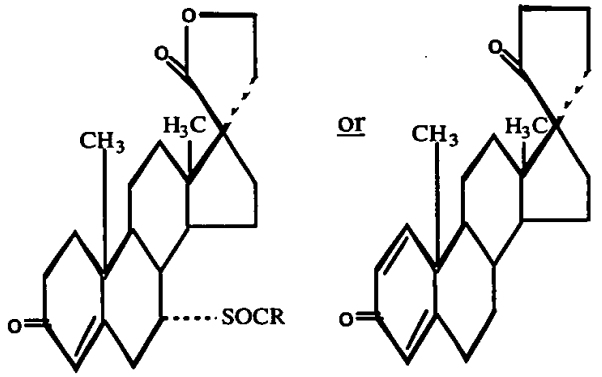
Specific compounds of interest within Formula II are the following:

1α-acetylthio-15β,16β-methylene-7α-methylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone; and

15β,16β-methylene-1α,7α-dimethylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone.

Methods to make the compounds of Formula II are described in U.S. Patent No. 4,789,668 to Nickisch et al. which issued 6 December 1988.

Yet another family of non-epoxy-steroidal compounds of interest is defined by a structure of Formula III:



(III)

wherein R is lower alkyl, with preferred lower alkyl groups being methyl, ethyl, propyl and butyl. Specific compounds of interest include:

3β,21-dihydroxy-17α-pregna-5,15-diene-17-carboxylic acid γ-lactone;

3β,21-dihydroxy-17α-pregna-5,15-diene-17-carboxylic acid γ-lactone 3-acetate;

3β,21-dihydroxy-17α-pregn-5-ene-17-carboxylic acid γ-lactone;

3β,21-dihydroxy-17α-pregn-5-ene-17-carboxylic acid γ-lactone 3-acetate;

21-hydroxy-3-oxo-17α-pregn-4-ene-17-carboxylic acid γ-lactone;

21-hydroxy-3-oxo-17α-pregna-4,6-diene-17-carboxylic acid γ-lactone;

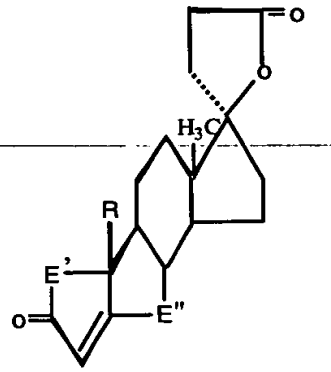
21-hydroxy-3-oxo-17α-pregna-1,4-diene-17-carboxylic acid γ-lactone;

7α-acylthio-21-hydroxy-3-oxo-17α-pregn-4-ene-17-carboxylic acid γ lactone; and

7α-acetylthio-21-hydroxy-3-oxo-17α-pregn-4-ene-17-carboxylic acid γ-lactone.

Methods to make the compounds of Formula III are described in U.S. Patent No. 3,257,390 to Patchett which issued 21 June 1966.

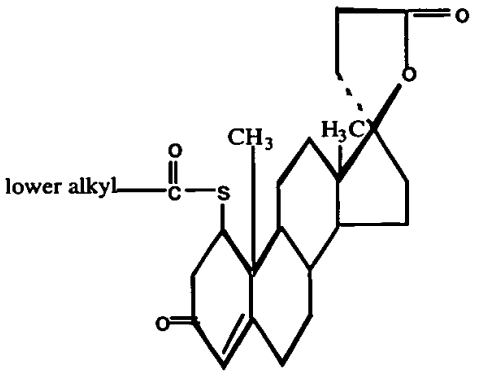
Still another family of non-epoxy-steroidal compounds of interest is represented by Formula IV:



(IV)

wherein E' is selected from the group consisting of ethylene, vinylene and (lower alkanoyl)thioethylene radicals, E" is selected from the group consisting of ethylene, vinylene, (lower alkanoyl)thioethylene and (lower alkanoyl)thiopropylene radicals; R is a methyl radical except when E' and E" are ethylene and (lower alkanoyl) thioethylene radicals, respectively, in which case R is selected from the group consisting of hydrogen and methyl radicals; and the selection of E' and E" is such that at least one (lower alkanoyl)thio radical is present.

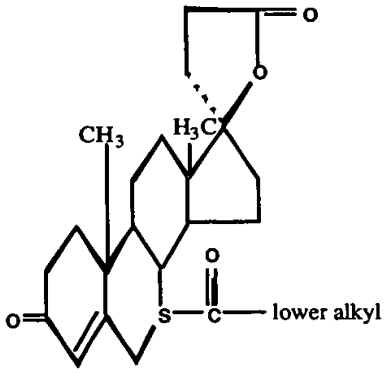
A preferred family of non-epoxy-steroidal compounds within Formula IV is represented by Formula V:



(V)

A more preferred compound of Formula V is 1-acetylthio-17α-(2-carboxyethyl)-17β-hydroxy-androst-4-en-3-one lactone.

Another preferred family of non-epoxy-steroidal compounds within Formula IV is represented by Formula VI:



(VI)

More preferred compounds within Formula VI include the following:

7α-acetylthio-17α-(2-carboxyethyl)-17β-hydroxy-androst-4-en-3-one lactone;

7β-acetylthio-17α-(2-carboxyethyl)-17β-hydroxy-androst-4-en-3-one lactone;

1α,7α-diacetylthio-17α-(2-carboxyethyl)-17β-hydroxy-androsta-4,6-dien-3-one lactone;

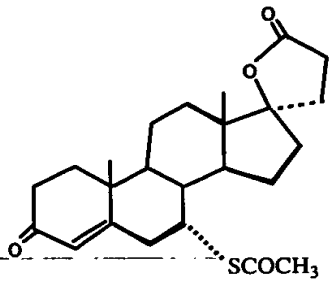
7α-acetylthio-17α-(2-carboxyethyl)-17β-hydroxy-androsta-1,4-dien-3-one lactone;

7α-acetylthio-17α-(2-carboxyethyl)-17β-hydroxy-19-norandrost-4-en-3-one lactone; and

7α-acetylthio-17α-(2-carboxyethyl)-17β-hydroxy-6α-methylandrost-4-en-3-one lactone;

In Formulae IV-VI, the term "alkyl" is intended to embrace linear and branched alkyl radicals containing one to about eight carbons. The term "(lower alkanoyl)thio" embraces radicals of the formula lower alkyl —C(=O)—S.

Of particular interest is the compound spironolactone having the following structure and formal name:



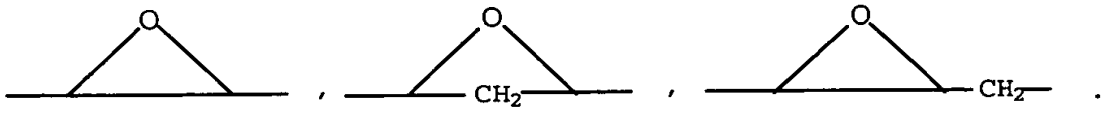
"spironolactone": 17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate.

Methods to make compounds of Formulae IV-VI are described in U.S. Patent No. 3,013,012 to Cella et al. which issued 12 December 1961. Spironolactone is sold by G.D. Searle & Co., Skokie, Illinois, under the trademark

"ALDACTONE", in tablet dosage form at doses of 25 mg, 50 mg and 100 mg per tablet.

Another group of aldosterone antagonists of particular interest in the present methods are epoxy-steroidal aldosterone antagonists and include a family of compounds having an epoxy moiety fused to the "C" ring of the steroidal nucleus. Especially preferred are 20-spiroxane compounds characterized by the presence of a 9α,11α-substituted epoxy moiety. Compounds 1 through 11, below, are illustrative 9α,11α-epoxy-steroidal compounds that may be used in the present methods. These epoxy steroids may be prepared by procedures described in Grob et al., U.S. Patent No. 4,559,332. Additional processes for the preparation of 9,11-epoxy steroidal compounds and their salts are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Epoxy-steroidal compounds suitable for use in the present invention consist of one or more of these compounds having a steroidal nucleus substituted with an epoxy-type moiety. The term "epoxy-type" moiety is intended to embrace any moiety characterized in having an oxygen atom as a bridge between two carbon atoms, examples of which include the following moieties:



epoxyethyl , 1,3-epoxypropyl , 1,2-epoxypropyl .

The term "steroidal", as used in the phrase "epoxy-steroidal", denotes a nucleus provided by a cyclopentenophenanthrene moiety, having the conventional "A", "B", "C" and "D" rings. The epoxy-type moiety may be attached to the cyclopentenophenanthrene nucleus at any attachable or substitutable positions, that is, fused to one of the rings of the steroidal nucleus or the moiety may be substituted on a ring member of the ring system. The phrase "epoxy-steroidal" is intended to

embrace a steroidal nucleus having one or a plurality of epoxy-type moieties attached thereto.

Epoxy-steroidal compounds suitable for use in the present invention include a family of compounds having an epoxy moiety fused to the "C" ring of the steroidal nucleus. Especially preferred are 20-spiroxane compounds characterized by the presence of a 9α,11α-substituted epoxy moiety. Table I, below, describes a series of 9α,11α-epoxy steroidal compounds which may be used in therapy. Especially preferred of these compounds of Table I is Compound #1 which is known by the common name epoxymexrenone and also by the USAN designation eplerenone. These epoxy steroids may be prepared by procedures described in U.S. Patent No. 4,559,332 to Grob et al issued 17 December 1985.

TABLE I: Aldosterone Receptor Antagonists

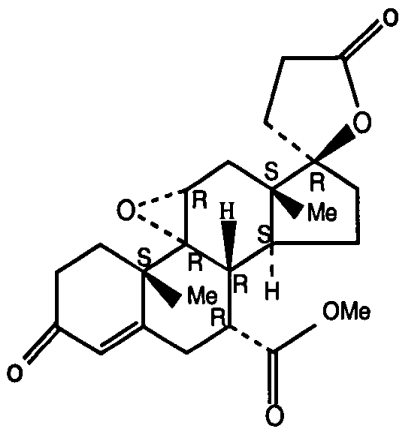
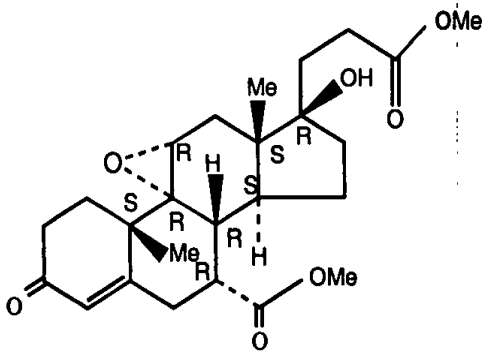
Compound #	Structure	Name
1		Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-,γ-lactone, methyl ester, (7α,11α,17β)-
2		Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-,dimethyl ester, (7α,11α,17β)-

TABLE I: Aldosterone Receptor Antagonist

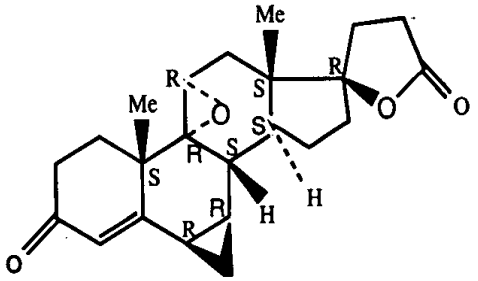
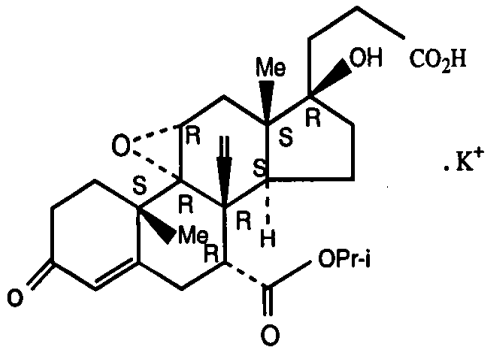
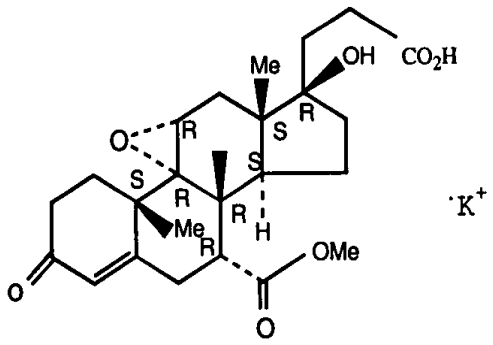
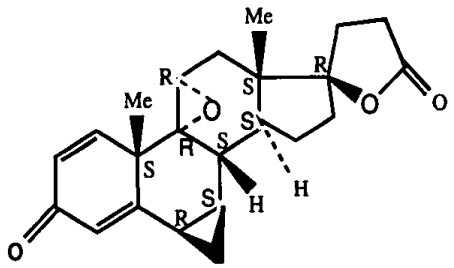
Compound #	Structure	Name
3		3'H-cyclopropa[6,7]pregna-4,6-diene-21-carboxylic acid, 9,11-epoxy-6,7-dihydro-17-hydroxy-3-oxo-, γ-lactone,(6β, 7β, 11α, 17β)-
4	 .K+	Pregn-4-ene-7,21-dicarboxylic acid,9,11-epoxy-17-hydroxy-3-oxo-,7-(1-methylethyl) ester, monopotassium salt,(7α,11α,17β)-

TABLE I: Aldosterone Receptor Antagonist

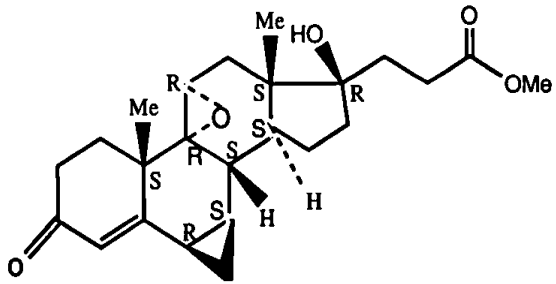
Compound #	Structure	Name
5		Pregn-4-ene-7,21-dicarboxylic acid,9,11-epoxy-17-hydroxy-3-oxo-,7-methylethyl) ester,monopotassium salt,(7α,11α,17β)-

6

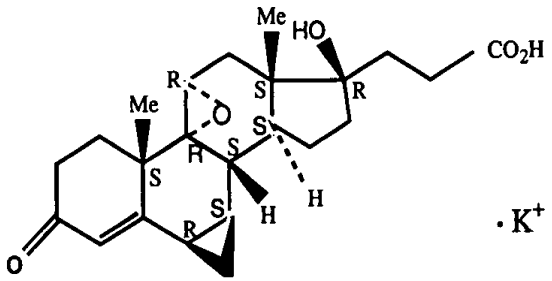


3'H-cyclopropa[6,7]pregna-1,4,6-triene-21-carboxylic acid,9,11-epoxy-6,7-dihydro-17-hydroxy-3-oxo-, γ-lactone(6β,7β,11α)-

TABLE I: Aldosterone Receptor Antagonist

Compound #	Structure	Name
7		3'H-cyclopropa[6,7]pregna-4,6-diene-21-carboxylic acid, 9,11-epoxy-6,7-dihydro-17-hydroxy-3-oxo-, methyl ester, (6β,7β,11α,17β)-

8



3'H-cyclopropa[6,7]pregna-4,6-diene-21-carboxylic acid, 9,11-epoxy-6,7-dihydro-17-hydroxy-3-oxo-, monopotassium salt, (6β,7β,11α,17β)-

TABLE I: Aldosterone Receptor Antagonist

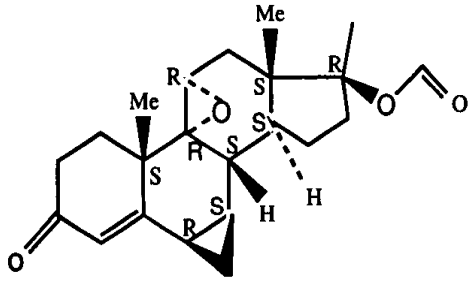
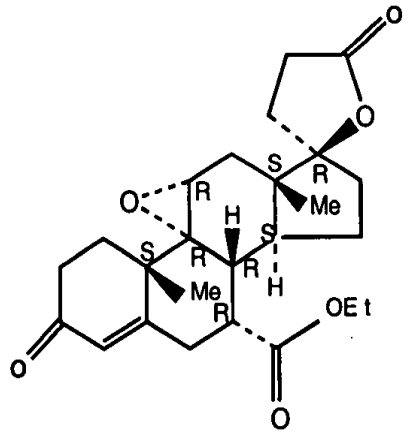
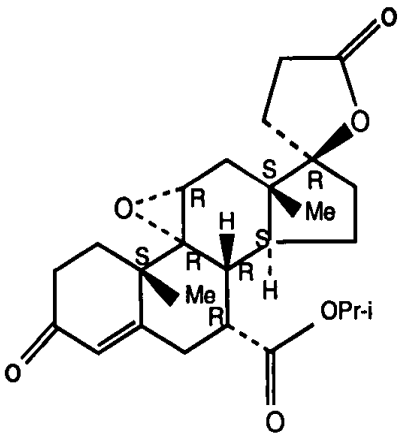
Compound #	Structure	Name
9		3'H-cyclopropa[6,7]pregna-1,4,6-triene-21-carboxylic acid, 9,11-epoxy-6,7-dihydro-17-hydroxy-3-oxo-,γ-lactone(6β,7β,11α,17β)-
10		Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-,γ-lactone, ethyl ester, (7α,11α,17β)-

TABLE I: Aldosterone Receptor Antagonist

Compound #	Structure	Name
11		Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-,γ-lactone, 1-methylethyl ester (7α,11α,17β)-

Administration may be accomplished by oral route, or by intravenous, intramuscular or subcutaneous injections. The formulation may be in the form of a bolus, or in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solution and suspensions may be prepared from sterile powders or granules having one or more pharmaceutically-acceptable carriers or diluents, or a binder such as gelatin or hydroxypropyl-methyl cellulose, together with one or more of a lubricant, preservative, surface-active or dispersing agent.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are tablets or capsules. A suitable daily dose for a mammal may vary widely depending on the condition of the patient and other factors. One ore more epoxy-steroidal compounds may be present in an amount of from about 1 to 400 mg, preferably from about 2 to 150 mg, depending upon the specific epoxy-steroidal compound selected and the specific disease state being targeted.

For disease states which require prevention, reduction or treatment of a cardiovascular disease state without incidence of hyperkalemia, for example, one or more epoxy-steroidal compounds, typically eplerenone, will be present in an amount in a range from about 5 mg to about 200 mg per dose. A preferred range for eplerenone would be from about 25 mg to 50 mg per dose. More preferably would be a range from about 10 mg to 15 mg per dose per day.mg per dose per day.

The active ingredients may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier.

The dosage regimen for treating a disease condition is selected in accordance with a variety of factors, including the type, age, weight, sex and medical condition of the patient, the severity of the disease, the route of

administration, and the particular compound employed, and thus may vary widely.

For therapeutic purposes, the active components of this invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the components may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain a controlled-release formulation as may be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The components may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Pharmaceutical compounds for use in the treatment methods of the invention may be administered in oral form or by intravenous adminstration. Oral administration of the therapy is preferred. Dosing for oral administration may be with a regimen calling for single daily dose, or for a single dose every other day, or for multiple, spaced doses throughout the day. The time period between the multiple ingestion steps may range from a few minutes to several hours, depending upon the properties of each active agent such a potency, solubility, bioavailability, plasma half-life and kinetic profile of the agent, as well as depending upon the age and condition of the patient. Examples of suitable pharmaceutically-acceptable formulations containing the active components for oral administration are given below.

Example 1

An oral dosage may be prepared by screening and then mixing together the following list of ingredients in the amounts indicated. The dosage may then be placed in a hard gelatin capsule.

Ingredients	Amounts
eplerenone	12.5 mg
magnesium stearate	10 mg
lactose	100 mg

Example 2

An oral dosage may be prepared by mixing together granulating with a 10% gelatin solution. The wet granules are screened, dried, mixed with starch, talc and stearic acid, screened and compressed into a tablet.

Ingredients	Amounts
eplerenone	12.5 mg
calcium sulfate dihydrate	100 mg
sucrose	15 mg
starch	8 mg
talc	4 mg
stearic acid	2 mg

Example 3

An oral dosage may be prepared by screening and then mixing together the following list of ingredients in the amounts indicated. The dosage may then be placed in a hard gelatin capsule.

Ingredients	Amounts
eplerenone	12.5 mg

magnesium stearate	10 mg
lactose	100 mg

Example 4

An oral dosage may be prepared by mixing together granulating with a 10% gelatin solution. The wet granules are screened, dried, mixed with starch, talc and stearic acid, screened and compressed into a tablet.

Ingredients	Amounts
eplerenone	12.5 mg
calcium sulfate dihydrate	100 mg
sucrose	15 mg
starch	8 mg
talc	4 mg
stearic acid	2 mg

Unit Dosages

Dosage unit forms of the pharmaceutical compounds may typically contain, for example, 0.5, 1, 5, 10, 20, 25, 37.5, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 mg of eplerenone. Preferred dosage unit forms contain about 1, 25, 50, 100, or 150 mg of eplerenone. The dosage unit form may be selected to accommodate the desired frequency of administration used to achieve the specified daily dosage. The amount of the unit dosage form of the pharmaceutical composition that is administered and the dosage regimen for treating the condition or disorder will depend on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the condition or disorder, the route and frequency of administration, and thus may vary widely.

It has been discovered, however, that the efficacy of the required daily dosage of the pharmaceutical compounds of the present invention does not appear to materially differ for once-a-day administration relative to twice-a-day administration with respect to the compounds described in this application. It is hypothesized that the compounds of the present invention deliver an amount of

eplerenone sufficient to inhibit a protracted genomic response caused by aldosterone binding to the aldosterone receptor site. Interruption of aldosterone binding by eplerenone prevents aldosterone-induced gene product synthesis resulting in an extended period of functional aldosterone receptor blockade that does not require a sustained plasma eplerenone concentration. Accordingly, once-a-day administration is preferred for such tablets for convenience of administration.

Form of Pharmaceutical Compounds

The pharmaceutical compounds of the present invention comprise eplerenone in association with one or more non-toxic, pharmaceutically-acceptable carriers, excipients and/or adjuvants (collectively referred to herein as "carrier materials"). The carrier materials must be acceptable in the sense of being compatible with the other ingredients of the composition and must not be deleterious to the recipient. The pharmaceutical compounds of the present invention may be adapted for administration by any suitable route by selection of appropriate carrier materials and a dosage of eplerenone effective for the treatment intended. For example, these compounds may be prepared in a form suitable for administration orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly (IM) or rectally.

Accordingly, the carrier material employed can be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose composition, for example, a tablet, which can contain from about 1% to about 95%, preferably about 10% to about 75%, more preferably about 20% to about 60%, and still more preferably about 20% to about 40%, by weight of eplerenone. Such pharmaceutical compounds of the invention can be prepared by any of the well known techniques of pharmacy, consisting essentially of admixing the components.

Sold State Forms Of Ep xy-Steroidal Aldoster ne Recept r Antagonists

The methods of the present invention encompass the administration of a therapeutically-effective amount of epoxy-steroidal aldosterone receptor antagonists, particularly eplerenone, in any of its solid state forms, either as one or more solid state forms per se or in the form of a pharmaceutical composition comprising one or more solid state forms of eplerenone. These novel solid state forms include, but are not limited to, solvated crystalline eplerenone, non-solvated crystalline eplerenone, and amorphous eplerenone.

In one embodiment, the eplerenone administered in accordance with the methods of the present invention is a non-solvated crystalline form of eplerenone having the X-ray powder diffraction pattern set forth in Table 1C below (referred to herein as the "higher melting point polymorph" or "Form H").

In another embodiment of the invention, the eplerenone administered in accordance with the methods of the present invention is a non-solvated crystalline form of eplerenone having the X-ray powder diffraction pattern set forth in Table 1D below (referred to herein as the "lower melting point polymorph" or "Form L").

Unformulated Form H exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic transition temperature as later discussed) than, for example, unformulated Form L. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form H, therefore, can provide an improved bioavailability profile relative to Form L. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

Form L possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form H. Solid state forms of eplerenone such as Form L that do not require the use of special processing or storage conditions, and that avoid the need

for frequent inventory replacement, are desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form L having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the eplerenone administered in accordance with the methods of the present invention is a solvated crystalline form of eplerenone. Preferably, the solvated crystalline forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form H and Form L typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure, the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form H and Form L.

In another embodiment of the invention, the eplerenone administered in accordance with the methods of the present invention is amorphous eplerenone. It is hypothesized that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form H and Form L.

In another embodiment, the eplerenone administered in accordance with the methods of the present invention is a combination comprising a first solid state form of eplerenone and a second solid state form of eplerenone. Generally, the first and second solid state forms of eplerenone are selected from Form H, Form L, solvated eplerenone and amorphous eplerenone. Such combinations may further comprise additional solid state forms of eplerenone and are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably at least about 1:1, still more preferably at least about 2:1, still more preferably at least about 5:1, and still more preferably at least about 9:1.

In another embodiment, the eplerenone is administered in the form of a pharmaceutical composition wherein the entire amount of eplerenone contained in the composition is present as phase pure Form H.

In another embodiment, the eplerenone is administered in the form of a pharmaceutical composition wherein the entire amount of eplerenone contained in the composition is present as phase pure Form L

In another embodiment, the eplerenone is administered in the form of a pharmaceutical composition wherein the entire amount of eplerenone contained in the composition is present as a phase pure solvated crystalline eplerenone.

In another embodiment, the eplerenone is administered in the form of a pharmaceutical composition wherein the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In another embodiment, the eplerenone is administered in the form of a pharmaceutical composition wherein the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, and the first and second solid state forms of eplerenone are selected from Form H, Form L, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9,

preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In another embodiment, the eplerenone is administered in the form of a pharmaceutical composition wherein the composition comprises both Form H and Form L. The ratio of the amount of Form L to Form H in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10; about 5:1 to about 1:5; about 2:1 to about 1:2; or about 1:1.

Although each of the above embodiments can embrace the administration of a solid state form of eplerenone over a broad range of eplerenone particle sizes, it has been discovered that coupling the selection of the solid state form of eplerenone with a reduction of the eplerenone particle size can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that solid state form of eplerenone.

In one such embodiment, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In another embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50 microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

In another such embodiment, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than

about 600 nm, and still more preferably less than about 400 nm. In another embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm. In another embodiment, the D_{90} particle size is between about 400 nm to about 800 nm.

Solid state forms of eplerenone having a particle size less than about 15 microns can be prepared in accordance with applicable particle size reduction techniques known in the art. Such techniques include, but are not limited to those described in U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001. U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001 are expressly incorporated by reference as if fully set forth at length. In accordance with the method of U.S. Patent 5,145,684, for example, particles of suitable size are prepared by dispersing the eplerenone in a liquid dispersion medium and wet-grinding the mixture in the presence of grinding media to reduce the particles to the desired size. If necessary or advantageous, the particles can be reduced in size in the presence of a surface modifier.

Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplerenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction

peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid eplerenone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation.

The term "D_{90} particle size" means the particle size of at least 90% of the particles as measured by such conventional particle size measuring techniques.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an effective amount of a Form H growth promoter and/or a Form L growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form H growth promoter and/or a Form L growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "T_m" means melting temperature.

The term "subject" as used herein refers to a mammal, preferably a human, who has been the object of treatment, observation or experiment.

The terms "treatment" and "treating" refers to any process, action, application, therapy, or the like, wherein a subject, including a human being, is provided medical aid with the object of improving the subject's condition, directly or indirectly, or slowing the progression of a condition or disorder in the subject.

The phrase "therapeutically-effective" qualifies the amount of the aldosterone antagonist that will achieve the goal of improvement in condition or disorder while avoiding adverse side effects typically associated with alternative therapies.

The term "pharmaceutically acceptable" is used adjectivally herein to mean that the modified noun is appropriate for use in a pharmaceutical product. Pharmaceutically acceptable cations include metallic ions and organic ions. More preferred metallic ions include, but are not limited to appropriate alkali metal salts, alkaline earth metal salts and other physiologically acceptable metal ions. Exemplary ions include aluminum, calcium, lithium, magnesium, potassium, sodium and zinc in their usual valences. Preferred organic ions include protonated tertiary amines and quaternary ammonium cations, including in part, trimethylamine, diethylamine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Exemplary pharmaceutically acceptable acids include without limitation hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulfonic acid, acetic acid, formic acid, tartaric acid, maleic acid, malic acid, citric acid, isocitric acid, succinic acid, lactic acid, gluconic acid, glucuronic acid, pyruvic acid, oxalacetic acid, fumaric acid, propionic acid, aspartic acid, glutamic acid, benzoic acid, and the like.

The phrase "aldosterone antagonist" embraces an agent or compound that counteract the effect of aldosterone. Such agents and compounds, such as mespirenone, may antagonize the action of aldosterone through pre-receptor mechanism. Other agents and compounds, such as eplerenone and spironolactone,

fall generally within a class known as aldosterone receptor antagonists and bind to aldosterone receptors such as are typically found in renal tubules, and prevent natural ligand activation of post-receptor events.

The terms "kidney" and "renal", when used in juxapposition to disease, means all manner of kidney dysfunctions. Such dysfunctions include, but are not limited to nephrosclerosis, impaired creatinine clearance, microalbuminuria, proteinuria, and end-stage renal disease.

The terms "heart," "cardiac" and "cardiovascular," when used in juxapposition to disease, means all manner of heart and vascular dysfunctions.

Characterization of Solid State Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form H and Form L, particularly with respect to the orientation of the ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-02 torsion angle.

In the Form H crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately –73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form L crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form H and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation,

the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide ring is increased relative to the distance determined for Form H. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form L in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer, samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1C, 1D and 1E set out the significant parameters of the main peaks in terms of 2q values and intensities for the Form H (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form L (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form H and Form L as a result of imperfections in the spacing of the crystal diffraction planes due to the route of manufacture of Form H and Form L (i.e. desolvation of a solvate). In addition, Form H is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical

purity (approximately 90%) of the Form H. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

Table 1C: FORM H DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1188	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.115	6.269	3121	19
14.438	6.13	15935	96.8
15.524	5.703	637	3.9
16.169	5.477	1349	8.2
16.699	5.305	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.66	5.018	6883	41.8
17.91	4.949	16455	100
18.379	4.823	3106	18.9
18.658	4.752	1216	7.4
19.799	4.48	1499	9.1
20.235	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1260	7.7
21.959	4.044	1279	7.8
22.461	3.955	4264	25.9
23.191	3.832	1026	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	686	4.2
26.868	3.316	912	5.5
27.093	3.288	1322	8
27.782	3.209	1236	7.5
28.34	3.147	1845	11.2
28.861	3.091	957	5.8
29.866	2.9892	745	4.5
30.627	2.9166	992	6

31.108	2.8726	1205	7.3
33.215	2.6951	1287	7.8
33.718	2.656	802	4.9
34.434	2.6024	914	5.6

Table 1D: FORM L DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11596	26.6
10.044	8.799	12048	27.6
11.206	7.889	4929	11.3
12.441	7.109	1747	4
12.752	6.936	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43646	100
15.46	5.727	2670	6.1
15.727	5.63	7982	18.3
16.016	5.529	3519	8.1
17.671	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.703	4.74	689	1.6
19.524	4.543	1126	2.6
20.103	4.413	3753	8.6
20.63	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.652	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.485	3.492	1702	3.9
25.707	3.463	2493	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.969	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.005	1211	2.8
30.173	2.9594	1506	3.5

30.584	2.9206	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8628	1068	2.4
31.605	2.8285	1038	2.4
32.059	2.7895	1211	2.8
32.64	2.7412	684	1.6
32.747	2.7324	758	1.7
33.46	2.6759	506	1.2
34.194	2.6201	1085	2.5
34.545	2.5943	915	2.1

Table 1E: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.151	8.707	2877	16.7
11.31	7.817	701	4.1
12.646	6.994	1027	5.9
13.193	6.705	15188	88
13.556	6.526	14225	82.4
14.074	6.287	1966	11.4
14.746	6.002	2759	16
15.165	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7980	46.2
17.28	5.127	17267	100
17.706	5.005	6873	39.8
18.555	4.778	545	3.2
18.871	4.699	1112	6.4
19.766	4.488	1704	9.9
20.158	4.401	1396	8.1
20.725	4.282	2644	15.3
21.787	4.076	1127	6.5
22.06	4.026	451	2.6
22.864	3.886	1542	8.9
23.412	3.796	14185	82.2
23.75	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.503	3.49	1736	10.1

25.761	3.455	1225	7.1
26.176	3.402	1346	7.8
26.548	3.355	1098	6.4
27.357	3.257	1944	11.3
27.605	3.229	2116	12.3
27.9	3.195	858	5
28.378	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.046	1182	6.8
29.679	3.008	2606	15.1
30.402	2.9377	2184	12.6
30.739	2.9063	648	3.8

Graphical examples of the x-ray diffraction patterns for Form H, Form L, and the methyl ethyl ketone solvate crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form H shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form L shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form H and Form L) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form L (approximate D_{90} particle size of about 180-450 microns) prepared by direct

crystallization from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form L (approximate D_{90} particle size of about 80-100 microns) (Form L prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form L, and milling the resulting Form L) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form H (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form L directly crystallized from methyl ethyl ketone, (b) non-milled Form L prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form L prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form H prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm or endotherms were associated with the melting/decomposition of Form L or Form H eplerenone.

4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form H and Form L) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed

in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form H, (b) Form L, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for eplerenone in the Form H, Form L, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form H and either Form L or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form H has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form L and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form H and Form L reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form H or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form L. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form H and Form L was seen in the C-H bending region. Form H has an absorption at approximately 1399 cm^{-1} which is not observed in Form L, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

Table 2

Absorption Region	Form H (cm^{-1})	Form L (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3,δCH2, δCH2(α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_sCH3	1380	1381	~1380	1378

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form H and Form L eplerenone are shown in Figs. 4 and 5, respectively. The Form H eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form L eplerenone. Form H is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm. Form L is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis of solvates was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute. An example of the thermogravimetry analysis profile for the methyl ethyl ketone solvate is shown in Fig. 6-A.

7. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form H, Form L, and several solvated crystalline forms.

Table 3A

Parameter	**Form H**	**Form L**	**Methyl ethyl ketone Solvate**
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
Z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calculated)	1.329 g/cm^3	1.275 g/cm^3	1.287 g/cm^3
R	0.0667	0.062	0.088

Table 3B

Parameter	**Acetone Solvate**	**Toluene Solvate**	**Butyl Acetate Solvate**[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
Z	4	4	4

Volume (Å)	2533.7	2596.6	2490.0
ρ (calculated)	1.239 g/cm^3	1.296 g/cm^3	1.334 g/cm^3
R	0.058	0.089	0.093

[1]The solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

Table 3C

Parameter	**Isobutyl Acetate Solvate[1]**	**Isopropanol Solvate[1]**	**Ethanol Solvate[1]**
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
Z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calculated)	1.337 g/cm^3	1.296 g/cm^3	1.234 g/cm^3
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell

parameters and molecular positions are substantially identical for most of the solvates tested.

Table 4

Solvent	**Stoichiometry (Solvent: Eplerenone)**	**Isostructural to Methyl Ethyl ketone Solvate?**	**Desolvation Temperature [1] (°C)**
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

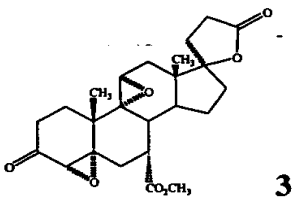
[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is

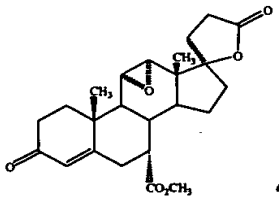
also reported in Table 4 above for a number of solvates. The unit cell of Form H is composed of four eplerenone molecules. The unit cell of Form L is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into Form H and/or Form L unit cells when the eplerenone molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

5. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form H during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone **3** (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α ,21-dicarboxylate, γ-lactone **4** (the "11,12-epoxide").



3

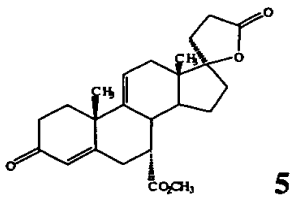


4

The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this

application.

Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone **5** (the "9,11-olefin") and Form H, it is hypothesized that the 9,11-olefin also can induce the formation of Form H during the desolvation of the solvate.



5

The diepoxide, 11,12-olefin and 9,11-olefin can be prepared as set forth, for example, in Examples 47C, 47B and 37H of Ng et al., WO98/25948, respectively.

A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 7, 8,and 10, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form H, suggesting that Form H and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form H. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form H, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

Table 5

Parameter	F rm H	Diepoxide	11,12 Epoxide	9,11 lefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
A	21.22 Å	21.328 Å	20.90 Å	20.90 Å
B	15.40 Å	16.16 Å	15.55 Å	15.74 Å
C	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
Z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm^3	1.349 g/cm^3	1.328 g/cm^3	1.279 g/cm^3
R	0.0667	0.0762	0.0865	0.0764

The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form H conformation. The relative ease of isolation of a Form H packing (directly from solution) for each impurity compound, indicates that the Form H lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

Preparati n of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this

solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally

favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry converstion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form L From Solvate

Form L eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof. If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form H and Form L. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form L, to reach

completion.

To ensure the preparation of a product that comprises substantially all Form L, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form L eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form L content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form L, preferably at least 50% Form L, more preferably at least 75% Form L, still more preferably at least 90% Form L, still more preferably at least about 95% Form L, and still more preferably substantially phase pure Form L.

2. Preparation of Form H From Solvate

A product comprising Form H can be prepared in substantially the same manner as set forth above for the preparation of Form L by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form H crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form H crystal formation during the desolvation of the solvate. The selected impurity generally is a Form H growth promoter or Form L growth inhibitor. It may be contained in the eplerenone starting material,

contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al. *J Amer Chem Soc* 1995;117:30 discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form H. The impurity preferably is a compound having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form H, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form H crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form H from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form H crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form H crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form H crystals.

A mixture of Form H and Form L is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form H in the product resulting from the initial desolvation of the solvate typically is less than

about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form L in the product.

Seeding

Form H crystals also can be prepared by seeding the solvent system with phase pure Form H crystals (or a Form H growth promoter and/or Form L growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form H in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form H seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form H seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form H crystals, particularly the preparation of Form H crystals by digestion as discussed below.

The Form H seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form H seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form H crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependent on the

eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80°C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form H is set forth below in Example 11.

The crystallized eplerenone product obtained using a Form H growth promoter or Form L growth inhibitor, and/or Form H seeding generally comprises at least 2% Form H, preferably at least 5% Form H, more preferably at least 7% Form H, and still more preferably at least about 10% Form H. The remaining crystallized eplerenone product generally is Form L.

Form H Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form H can be prepared by suitable grinding eplerenone. Concentrations of Form H in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form L From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form H and Form L. A product having a greater Form L content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form H by seeding the solvent system with phase pure Form L crystals, or by using a Form L growth promoter and/or Form H growth

inhibitor. The seeding protocol and the weight ratio of the amount of Form L seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form H eplerenone by seeding with phase pure Form H crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form L, preferably at least 50% Form L, more preferably at least 75% Form L, more preferably at least 90% Form L, still more preferably at least about 95% Form L, and still more preferably substantially phase pure Form L.

The seeding protocols described in this section and in the prior section relating to the preparation of Form H eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form L Directly From Solution

Form L eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form L eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form L eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled

until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form L, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the

solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form L crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form L crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form L eplerenone, (ii) vapor diffusion growth of Form L eplerenone, (iii) isolation of Form L eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form L eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

5. Preparation of Form H Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form H and Form L, particularly if Form H growth promoters or Form L growth inhibitors are present or the solvent is seeded with phase pure Form H crystals, Form H should crystallize directly from solution since Form H is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form H growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

6. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form H and Form L of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the solvate as previously described yields either Form H or Form L eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals. A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested

will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form L is desired, a high purity eplerenone starting material typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form L, preferably at least 50% Form L, more preferably at least 75% Form L, more preferably at least 90% Form L, still more preferably at least about 95% Form L, and still more preferably substantially phase pure Form L.

If a product comprising primarily or exclusively Form H is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form H growth promoter and/or Form L growth inhibitor as is needed to yield Form H. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form H, preferably at least 50% Form H, more preferably at least 75% Form H, more preferably at least 90% Form H, still more preferably at least about 95% Form H, and still more preferably substantially phase pure Form H.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of eplerenone. These processes are illustrated in Examples 17 and 18 below.

W rking Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordance with scheme 1 set forth in Ng et al., WO98/25948.

Example 5: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form L crystalline eplerenone from resulting solvate.

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form L crystalline eplerenone:

The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient pressure. The dried solid was determined to be pure Form L by DSC and XPRD analysis.

Example 6: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 5. Form L eplerenone was formed from each of the solvates substantially as described in Step B of Example 5.

Example 7: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane vapor allowed to diffuse into the methyl ethyl ketone solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 8: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting

solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

Example 9: Slurry Conversion

Approximately 150 mg of Form L eplerenone and 150 mg of Form H eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form L eplerenone.

Example 10: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form H crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables 6A and 6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form H content by monitoring the area of the Form H

diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

Table 6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

Table 6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 10 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form H or the

diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 11 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form H was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form H was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form H diffraction peak at about 12.1 degrees two theta and the estimated Form H content for each sample are given in Table 6C below.

Table 6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form H Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form H
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table 6C confirm that the presence of the diepoxide affects the formation of Form H during the desolvation. These results indicate that the diepoxide is effective in inducing the formation of Form H eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form H formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a

higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 12 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form H in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

A. 11,12-Epoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form H or the 11,12-epoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form H was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0, 1% and 5%. Form H was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form H diffraction peak at 12.1 degrees two theta and estimated Form H content for each sample are given in Table 6D.

Table 6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form H Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form H
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table 6D confirm that the presence of the 11,12-epoxide impacts the formation of Form H during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form H eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

Example 11: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with Form H seeding, and (iv) crystallization of low purity eplerenone with Form L seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled eplerenone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of eplerenone were analyzed and blended to yield a material that was

61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45° C and low endpoint was defined as 5°C. High cooling rate was defined as 3° C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketone were charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

Form H Seeding

In the Form H seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form H crystals to initiate crystallization. The Form H seed crystals were prepared by the digestion process described in Example 13 below.

Form L Seeding

In the Form L seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form L crystals to initiate crystallization. The Form L seed crystals were prepared by the crystallization and desolvation process described in Example 5 above.

Results from the experiments are reported in Table 7A. In the n+1 crystallization experiment, Form H was detected only in the experiments employing

low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form H when analyzed by X-ray powder diffraction.

The Form H seeding experiment (where high purity eplerenone was seeded with Form H) yielded a product that was 77% Form H based on X-ray powder diffraction analysis, but entirely Form H based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form H. This experiment was the only one of the four experiments of this Example where Form H was created in the absence of the diepoxide.

The Form L seeding experiment (where low purity eplerenone was seeded with Form L) yielded a product that was entirely Form L.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

Table 7A

Cooling Rate[1]	Cooling Endpoint[2]	Impurity Level[3]	Nucleation Temperature (°C)	Weight Percent 11,12-Epoxide[4]	Weight Percent USDiepoxide[4]	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form H (XPRD)
+	+	-	57.0	ND	ND	100.3	66.1	ND
+	-	-	54.9	ND	ND	100.3	98.1	ND
-	+	-	60.9	ND	ND	100.3		ND
-	-	-	63.4	ND	ND	100.5	79.3	ND
+	+	++	N/A	4.8	36.6	43.3	27	100[5]
+	+	+	52.2	0.49	0.88	98.3	62	29
+	-	+	53.3	0.56	1.0	98.1	87	9
0	0	0	59.0	0.18	0.36	99.4	75	5
-	+	+	63.3	0.20	0.44	99.4	36	31
-	-	+	61.4	0.18	0.40	99.5	87	ND
0	0	0	60.6	0.18	0.36	99.5	79.2	ND
0	0	0	55.9	0.38	0.80	98.6	80.5	<3%
0	0	100.8% eplerenone seeded with Form H		0.03	ND	100.4	82.2	77/100[6]
0	0	89.3% eplerenone seeded with Form L		0.33	0.50	97.5	80.2	ND

[1]Cooling Rate: (+) = 3°C/min.; (0) = 1.5 °C/min.; and (-) = 0.1 °C/min.
[2]Cooling Endpoint: (+) = 45°C; (0) = 25 °C; and (-) = 5°C.
[3]Impurity Level: : (+) = 89.3% purity eplerenone starting material; (++) = 61.1% purity eplerenone starting material; (0) = 100.8% purity eplerenone starting material; and (-) = 94.5% purity eplerenone starting material.
[4]Weight percent after drying solvate in a vacuum oven at 75°C.
[5]Appears to be mixture of Form H and diepoxide when analyzed by XPRD.
[6]Appears to be 77% Form H when analyzed by XPRD and 100% Form H when analyzed by DSC

A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table 7A is shown in Fig. 15. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 16 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 17 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

Form H Content

A cube plot of Form H weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table 7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form H. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form H although some Form H may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 19 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form H in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form H content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form H content. No Form H resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form H was present in both cases, with significantly more Form H at higher endpoint temperatures.

Table 7B reports the weight fraction of Form H measured in materials dried using either a fluid bed (LAB-LINE/P.R.L. Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form H content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

Table 7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form H
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

Example 12: Crystallization of a Mixture of Form H and Form L From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form L

Form H eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C. The drying was completed in the vacuum oven at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form L crystalline structure.

Example 13: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form H

Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of

the solvent turnover, the mixture was cooled to 25°C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form H eplerenone.

Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form H by XPRD.

Example 14: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form L

Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form L (no Form H detected).

Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone

(presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form L with no diffraction peaks for Form H present.

Example 15: Crystallization of Form L Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved, the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form L eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form L eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids

were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form L crystals.

Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form L eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to –5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form L eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to –5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 16 hours. The dried solids were determined to be pure Form L eplerenone by DSC and XPRD analysis.

Example 16: Crystallization of Form H Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form H and Form L. The crystals

were translucent by microscopy, indicating that desolvation (and conversion to either Form H or Form L) did not occur.

Example 17: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form L crystalline eplerenone.

Example 18: Preparation of Amorphous By Lyophilization

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes. Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 21 and 22 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 21 is attributable to the aluminum sample container.

Example 19: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form L eplerenone are prepared and have the following composition:

Ingredient	**Weight % f Tablet**
Form L Eplerenone	29.41
Form H Eplerenone	Not Detected

Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

Example 20: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	**Amount (mg)**
Form L Eplerenone	90.0
Form H Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 21: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	**Amount (mg)**
Form L Eplerenone	190.0
Form H Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0

Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 22: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

The following examples illustrate aspects of the present invention but should not be construed as limitations. The experimental procedures used to generate the data shown are discussed in more detail below. The symbols and conventions used in these examples are consistent with those used in the contemporary pharmacological literature. Unless otherwise stated, (i) all percentages recited in these examples are weight percents based on total composition weight, (ii) total composition weight for capsules is the total capsule fill weight and does not include the weight of the actual capsule employed, and (iii) coated tablets are coated with a conventional coating material such as Opadry White YS-1-18027A and the weight fraction of the coating is about 3% of the total weight of the coated tablet.

Example 23: 25 Mg Dose Immediate Release Tablet

A 25 mg dose immediate release tablet (tablet diameter of 7/32") was prepared having the following composition:

Table 8

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	29.41	25.00
Lactose Monohydrate (#310, NF)	42.00	35.70

Microcrystalline Cellulose (NF, Avicel PH101)	18.09 (7.50% intragranular plus 10.59% extragranular)	15.38
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00	4.25
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00	2.55
Sodium Lauryl Sulfate (NF)	1.00	0.85
Talc (USP)	1.00	0.85
Magnesium Stearate (NF)	0.50	0.42
Total	100	85
Opadry White YS-1-18027A	3.00	2.55

Example 24: 50 Mg Dose Immediate Release Tablet

A 50 mg dose immediate release tablet (tablet diameter of 9/32") was prepared having the following composition:

Table 9

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	29.41	50.00
Lactose Monohydrate (#310, NF)	42.00	71.40
Microcrystalline Cellulose (NF, Avicel PH101)	18.09 (7.50% intragranular plus 10.59% extragranular)	30.75

Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00	8.50
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00	5.10
Sodium Lauryl Sulfate (NF)	1.00	1.70
Talc (USP)	1.00	1.70
Magnesium Stearate (NF)	0.50	0.85
Total	100	170
Opadry White YS-1-18027A	3.00	5.10

Example 25: 100 Mg Dose Immediate Release Tablet

A 100 mg dose immediate release tablet formulation (tablet diameter of 12/32") was prepared having the following composition:

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	29.41	100.00
Lactose Monohydrate (#310, NF)	42.00	142.80
Microcrystalline Cellulose (NF, Avicel PH101)	18.09 (7.50% intragranular plus 10.59% extragranular)	61.50
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00	17.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00	10.20

Sodium Lauryl Sulfate (NF)	1.00	3.40
Talc (USP)	1.00	3.40
Magnesium Stearate (NF)	0.50	1.70
Total	100	340
Opadry White YS-1-18027A	3.00	10.20

Example 26: 10 mg Dose Immediate Release Capsule

A 10 mg dose immediate release capsule formulation was prepared having the following composition:

Table 10

INGREDIENT	AMOUNT (mg)	REPRESENTATIVE BATCH AMOUNT (kg)
Eplerenone	10.0	1.00
Lactose, Hydrous NF	306.8	30.68
Microcrystalline Cellulose, NF	60.0	6.00
Talc, USP	10.0	1.00
Croscarmellose Sodium, NF	8.0	0.80
Sodium Lauryl Sulfate, NF	2.0	0.20
Colloidal Silicon Dioxide, NF	2.0	0.20
Magnesium Stearate, NF	1.2	0.12

Total Capsule Fill Weight	400.0	40.00
Hard Gelatin Capsule, Size #0, White Opaque	1 Capsule	100,000 Capsules

Example 27: 25 mg Dose Immediate Release Capsule

A 25 mg dose immediate release capsule formulation was prepared having the following composition:

Table 11

INGREDIENT	AMOUNT (mg)	REPRESENTATIVE BATCH AMOUNT (kg)
Eplerenone	25.0	2.50
Lactose, Hydrous NF	294.1	29.41
Microcrystalline Cellulose, NF	57.7	5.77
Talc, USP	10.0	1.00
Croscarmellose Sodium, NF	8.0	0.80
Sodium Lauryl Sulfate, NF	2.0	0.20
Colloidal Silicon Dioxide, NF	2.0	0.20
Magnesium Stearate, NF	1.2	0.12
Total Capsule Fill Weight	400.0	40.00
Hard Gelatin Capsule, Size #0, White Opaque	1 Capsule	100,000 Capsules

Example 28: 50 mg Dose Immediate Release Capsule

A 50 mg dose immediate release capsule formulation was prepared having the following composition:

Table 12

INGREDIENT	AMOUNT (mg)	REPRESENTATIVE BATCH AMOUNT (kg)
Eplerenone	50.0	5.00
Lactose, Hydrous NF	273.2	27.32
Microcrystalline Cellulose, NF	53.6	5.36
Talc, USP	10.0	1.00
Croscarmellose Sodium, NF	8.0	0.80
Sodium Lauryl Sulfate, NF	2.0	0.20
Colloidal Silicon Dioxide, NF	2.0	0.20
Magnesium Stearate, NF	1.2	0.12
Total Capsule Fill Weight	400.0	40.00
Hard Gelatin Capsule, Size #0, White Opaque	1 Capsule	100,000 Capsules

Example 29: 100 mg Dose Immediate Release Capsule

A 100 mg dose immediate release capsule formulation was prepared having the following composition:

Table 13

INGREDIENT	AMOUNT (mg)	REPRESENTATIVE BATCH AMOUNT

		(kg)
Eplerenone	100.0	10.00
Lactose, Hydrous NF	231.4	23.14
Microcrystalline Cellulose, NF	45.4	4.54
Talc, USP	10.0	1.00
Croscarmellose Sodium, NF	8.0	0.80
Sodium Lauryl Sulfate, NF	2.0	0.20
Colloidal Silicon Dioxide, NF	2.0	0.20
Magnesium Stearate, NF	1.2	0.12
Total Capsule Fill Weight	400.0	40.00
Hard Gelatin Capsule, Size #0, White Opaque	1 Capsule	100,000 Capsules

Example 30: 200 mg Dose Immediate Release Capsule

A 200 mg dose immediate release capsule formulation was prepared having the following composition:

Table 14

INGREDIENT	AMOUNT (mg)	REPRESENTATIVE BATCH AMOUNT (kg)
Eplerenone	200.0	20.00
Lactose, Hydrous NF	147.8	14.78
Microcrystalline Cellulose, NF	29.0	2.90
Talc, USP	10.0	1.00
Croscarmellose Sodium, NF	8.0	0.80

Sodium Lauryl Sulfate, NF	2.0	0.20
Colloidal Silicon Dioxide, NF	2.0	0.20
Magnesium Stearate, NF	1.2	0.12
Total Capsule Fill Weight	400.0	40.00
Hard Gelatin Capsule, Size #0, White Opaque	1 Capsule	100,000 Capsules

Example 31

Clinical Study in Patients with Heart Disease

Patients: One thousand six hundred and sixty-three (1,663) patients with severe heart failure were enrolled in the study. Patients were eligible for enrollment in the study if they had a history of New York Heart Association (NYHA) Class IV heart failure within 6 months but no less than 6 weeks from randomization, and were NYHA Class III or IV at the time of enrollment. Eligible patients had a left ventricular ejection fraction of ≤35 percent and were to be receiving treatment with an angiotensin-converting enzyme inhibitor, if tolerated, and a loop diuretic. Treatment with digitalis and vasodilators was allowed, but potassium-sparing diuretics were not permitted. Oral potassium supplements were not recommended unless hypokalemia (serum potassium <3.5 mmol per liter) developed. A low salt diet (100-200 mEg/day, sodium) was recommended to all patients. Patients were excluded from the trial if they had clinically significant operable valvular disease (other than mitral or tricuspid regurgitation), congenital heart disease, unstable angina, primary hepatic failure, active malignancy, a heart transplant or were a candidate for heart transplantation, or any life threatening diseae (other than heart failure). Other criteria for exclusion were a serum creatinine concentration >2.5 mg per deciliter (>220 μmol per liter) or a serum potassium concentration >5.0 mmol per liter. The protocol was approved by the

Institutional Review Boards or Ethics Committees of all participating institutions. Written informed consent was obtained from all patients.

Study Group: Subjects were randomly assigned to receive either spironolactone 25 mg daily or placebo. HRQOL was assessed in a subsample of 90 subjects in 2 participating countries using the Medical Outcomes Trust Short-Form 36-item Health Survey (SF-36). Assessments were scheduled at baseline, 1, 2, 3, and 6 months after initiation of therapy.

HRQOL Evaluation: HRQOL was assessed using the Short-Form 36-item Health Survey (SF-36). (Ware JE, Snow KK, Kosinski M, Gandek B. SF-36 Health Survey: Manual and Interpretation Guide. Boston, MA: The Health Institute, 1993). Assessments were scheduled at baseline, 1, 2, 3, 6 and 12 months after initiation of therapy. The analysis presented herein is limited to 6-month follow-up as less than 50% of subjects had 12-month follow-up data.

Statistical Analyses: Change from baseline was assessed for the 8 dimension scores of the SF-36, as well as the Physical (PCS) and Mental Composite Summary (MCS) scores. (Ware JE, Kosinski M, Keller SD. SF-36 Physical and Mental Health Summary Scales: A User's Manual. Boston, MA: The Health Institute, 1994). The PCS and MCS are generated to represent weighted summaries scores of the four physical functioning scales and four mental functioning scales of the SF-36. The summary scores are generally regarded as having improved reliability and precision as they are based on an incrementally larger number of health states contained in the SF-36 items. (Ware JE, Kosinski M, Keller SD. SF-36 Physical and Mental Health Summary Scales: A User's Manual. Boston, MA: The Health Institute, 1994.) Changes from baseline scores were compared between groups using non-directional independent groups t-tests. An alpha level of 0.05 was considered to be statistically significant. As an indicator of the responsiveness of the SF-36 scales to changes in health-related quality of life in severe heart failure

patients, effect size estimates were also calculated. Effect size estimates were calculated as [mean (spironolactone) – mean (placebo)] / (SD placebo at baseline). (Hays RD, Anderson R, Revicki D. Psychometric considerations in evaluating health-related quality of life. *Quality of Life Research* 1993;2:441-449; Samsa G, Edelman D, Rothman ML, et al. Determining clinically important differences in health status measures. A general approach with illustrations to the Health Utilities Index Mark II. *PharmacoEconomics*1999;15:141-155). All analyses were carried out using SAS System for Windows, Release 6.12. (SAS version 6.12, SAS Institute Inc., North Carolina, 1996).

Patient Characteristics: Baseline HRQOL was assessed in a subsample of 90 subjects in 2 participating countries (i.e., Brazil and Canada). Forty-six subjects were randomized to spironolactone; 44 were randomized to the placebo arm. Sixty subjects had complete data for the 6 months of follow-up. The characteristics of the patients who completed 6 months of observations for the HRQOL study are presented in Table 15.

Table 15

Baseline Characteristics

Characteristic	Placebo (N=28)	Spironolactone (N=32)
Age (yr)	58.3 ± 10.2	61.0± 12.1
Race (%)		
Caucasian	25 (89%)	22 (69%)
Black	3 (11%)	9 (28%)
Other	0 (0%)	1 (3%)
Gender (no., %)		

Male	18 (64%)	22 (69%)
Female	10 (36%)	10 (31%)
Heart Failure Cause (no., %)		
Ischemic	10 (36%)	11 (34%)
Nonischemic	18 (64%)	21 (66%)
Left Ventricular Ejection Fraction (%)	26.3 ± 6.6	26.9 ± 6.2
New York heart Association Class (no., %)		
III	21 (75%)	19 (60%)
IV	7 (25%)	13 (40%)

No significant differences were observed between the active treatment and placebo groups in demographics or SF-36 scores at baseline (Table 16).

Table 16.

Baseline SF-36 Scores

SF-36 Dimension	Placebo* (N=28)	Spironolactone* (N=32)
Physical Functioning	33.3± 18.5	27.0 ± 21.2
Role Limitations – Physical	23.2± 20.7	14.1± 21.0
Bodily Pain	65.5± 26.5	57.7± 28.8
General Health	48.2± 19.2	38.4± 22.1
Vitality	38.0± 19.7	28.9± 23.3
Role Limitations – Emotional	25.9± 28.2	25.0± 34.9

Social Functioning	54.6± 25.3	45.7± 28.2
Mental Health	56.2± 22.0	44.3± 24.3
SF-36 Summary Scores		
Physical Composite Summary (PCS)	35.5± 6.3	32.5± 6.3
Mental Composite Summary (MCS)	39.5± 9.1	35.4± 11.7

* Figures are mean ± standard deviation

Drop Outs: The 30 subjects (14 spironolactone / 16 placebo) not completing the 6 months of follow-up did not differ from the remaining subjects in terms of age, gender, HF etiology. A larger proportion of patients not completing the 6 month follow-up were in NYHA Class IV at baseline (56.7%) compared to those with complete follow-up (33.3%; p=0.042). However, the distribution of NYHA Class IV was not significantly different between the spironolactone and placebo arms, with 64.3% of patients randomized to spironolactone and not completing 6 months of follow-up, compared to 50.0% in the placebo arm.

Effect of Spironolactone on HRQOL: Statistically significant changes from baseline for all 8 SF-36 dimension scores in the spironolactone arm, compared to only 6 dimensions in the placebo arm, were observed at 3 and 6 months (Table 17). At Month 3, the spironolactone treated group had significantly greater improvements (mean ± standard deviation) in *Mental Health* (spironolactone = 19.9 ± 21.1 versus placebo = 3.1 ± 20.9; p=0.004) and MCS (spironolactone = 13.2 ± 11.8 versus placebo = 5.3 ± 11.6; p=0.016) scores compared to the placebo group.

The positive impact of spironolactone treatment on change from baseline for *Mental Health* subscale scores continued to be statistically significant at 6 months (spironolactone = 17.5 ± 22.9 versus placebo = 4.5 ± 25.7; p=0.044). The trend toward beneficial effects on overall MCS was still apparent at 6 months (spironolactone = 10.3 ± 12.8 versus placebo = 4.5 ± 14.0), but the difference was not statistically significant (p=0.418).

Table 17

Change from Baseline in SF-36 Scores at 3 and 6 months

	3 Months		6 Months	
SF-36 Dimension	Placebo	Spironolactone	Placebo	Spironolactone
Physical Functioning	12.1± 21.0[a]	7.9± 19.4[a]	13.5± 22.8[a]	11.4± 21.5[a]
Role Limitations – Physical	16.4± 37.7[a]	23.3± 40.1[a]	18.5± 37.7[a]	14.8± 32.3[a]
Bodily Pain	8.9± 25.7	19.6± 30.0[a]	8.4± 32.7	15.9± 30.5[a]
General Health	9.3± 18.7[a]	9.7± 20.4[a]	10.4± 19.5[a]	12.0± 22.3[a]
Vitality	18.2± 24.3[a]	16.7± 24.1[a]	16.5± 23.0[a]	19.0± 25.5[a]
Role Limitations – Emotional	24.4± 47.7[a]	37.8± 46.1[a]	35.8± 46.2[a]	24.0± 39.9[a]
Social Functioning	18.1± 31.8[a]	24.6± 33.6[a]	18.1± 31.8[a]	23.1± 32.1[a]
Mental Health	3.1± 20.9	19.9± 21.2[a,b]	4.5± 25.7	17.5± 22.9[a,b]
SF-36 Summary Scores				
Physical Composite Summary (PCS)	4.4± 6.1[a]	3.0± 8.1[a]	4.4± 7.7[a]	3.9± 9.1[a]
Mental Composite Summary (MCS)	5.3± 11.6[a]	13.2± 11.8[a,b]	4.5± 14.0[a]	10.3± 12.8[a]

a – $p < 0.05$ for within group change from baseline

b – $p < 0.05$ for between group comparison of change from baseline.

Effect size statistics: Effect size statistics for the SF-36 scale and summary scores at months 3 and 6 ranged in magnitude from 0.03 to 0.86 (Table 18). As with the results of the statistical comparisons, the largest effect size estimates were for the changes in *Mental Health* and the MCS at 3 months (0.76 and 0.86, respectively). The effect size statistics were the smallest for the physical dimensions of the SF-36 in these patients.

Table 18.

Effect Size Estimates for Change in SF-36 Scores

SF-36 Dimension	3 Months	6 Months
Physical Functioning	-0.23	-0.11
Role Limitations – Physical	0.33	-0.18
Bodily Pain	0.40	0.28
General Health	0.03	0.08
Vitality	-0.07	0.13
Role Limitations – Emotional	0.48	-0.42
Social Functioning	0.26	0.20
Mental Health	0.76	0.59
SF-36 Summary Scores		
Physical Composite Summary (PCS)	-0.21	-0.07
Mental Composite Summary (MCS)	0.86	0.64

Figures are effect size estimates, calculated as e.s. = [mean (spironolactone) – mean (placebo)] / (SD placebo at baseline)

Results: Sixty subjects (32 – spironolactone, 28 – placebo) had complete data for 6 months of follow-up. No significant differences were observed between active treatment and placebo in the SF-36 scores at baseline. At 3 months, the spironolactone treated group had significantly greater improvements in *Mental Health* (spironolactone = 19.9 ± 21.2 versus placebo = 3.1 ± 20.9; p=0.004) and Mental Composite Summary (spironolactone = 13.2 ± 11.8 versus placebo = 5.3 ± 11.6; p=0.016) scores compared to the placebo group. The positive impact of spironolactone treatment on change from baseline for *Mental Health* subscale

scores continued to be statistically significant at 6 months (spironolactone = 17.5 ± 22.9 versus placebo = 4.5 ± 25.7; p=0.044).

Conclusion: The addition of the aldosterone receptor antagonist spironolactone to conventional heart failure therapy appears to positively impact self-reported HRQOL in subjects with severe HF. In particular, in this small sample of severe HF, the addition of spironolactone therapy appeared to have a strong, positive impact on mental health status, with no detrimental effect on physical health or functioning.

Example 32

CLINICAL PROTOCOL FOR A double-blind, randomized, PLACEBO-controlled TRIAL EVALUATING THE SAFETY AND EFFICACY OF EPLERENONE IN PATIENTS WITH HEART FAILURE FOLLOWING ACUTE MYOCARDIAL INFARCTION

Summary

The objective of this trial is to compare the effect of eplerenone plus standard therapy versus placebo plus standard therapy on the rate of all cause mortality in patients with heart failure (HF) after an acute myocardial infarction (AMI). Secondary endpoints include cardiovascular morbidity and mortality and quality of life. Quality of life includes mood, depression, anxiety, mental health status, and all parameters relevant to cognitive function as assessed by The Kansas City Cardiomyopathy Questionnaire (KCCQ), the Short Form - 12 Health Survey (SF-12), the EuroQoL Health Rating Scale, the Medical Outcomes Study Depression Scale (MOS-D), and Brief Symptom Inventory-Anxiety (BSI-A).

This multicenter, randomized, double-blind, placebo-controlled, two-arm, parallel group trial will continue until 1,012 deaths occur, which is estimated to require

approximately 6,200 randomized patients followed for an average of approximately 2.5 years.

Patients eligible for this study will have:

- AMI (the index event) documented by:
  - abnormal cardiac enzymes (creatine phosphokinase [CPK] >2 x upper limit of the normal range [ULN] and/or CPK-MB >10% of total CPK); and
  - an evolving electrocardiogram (ECG) diagnostic of MI (progressive changes in ST segment and T wave compatible with AMI with or without presence of pathological Q waves), AND
- left ventricular (LV) dysfunction, demonstrated by LV ejection fraction (LVEF) ≤40% determined following AMI and before randomization, AND
- clinical evidence of HF documented by at least one of the following:
  - pulmonary edema (bilateral posttussive crackles extending at least 1/3 of the way up the lung fields in the absence of significant chronic pulmonary disease); OR
  - chest x-ray showing pulmonary venous congestion with interstitial or alveolar edema; OR
  - auscultatory evidence of a third heart sound (S_3) with persistent tachycardia (>100 beats per minute).

Patients will receive standard therapy which may include angiotensin converting enzyme (ACE) inhibitors, diuretics, nitrates, and β-blockers, and may have received anticoagulants and antiplatelet agents, and may have received thrombolytics or emergency angioplasty.

Eligible patients may be identified for inclusion at any time following emergency room evaluation and presumptive diagnosis of AMI with HF. Patients who qualify for this study will be randomized between 3 (>48 hours) and 10 days post-AMI if their clinical status is stable, e.g., no vasopressors, inotropes, intra-

aortic balloon pump, hypotension (systolic blood pressure [SBP]<90 mmHg), or recurrent chest pain likely to lead to acute coronary arteriography. Patients with implanted cardiac defibrillators are excluded.

Patients will be randomized to receive eplerenone 25 mg QD (once daily) or placebo. At four weeks, the dose of study drug will be increased to 50 mg QD (two tablets) if serum potassium <5.0 mEq/L. If at any time during the study the serum potassium is >5.5 mEq/L but <6.0 mEq/L, the dose of study drug will be reduced to the next lower dose level, i.e., 50 mg QD to 25 mg QD (one tablet), 25 mg QD to 25 mg QOD (every other day), or 25 mg QOD to temporarily withheld. If at any time during the study the serum potassium is ≥6.0 mEq/L, study medication should be temporarily withheld, and may be restarted at 25 mg QOD when serum potassium is <5.5 mEq/L. If at any time during the study the serum potassium is persistently ≥6.0 mEq/L, study medication should be permanently discontinued. If the patient becomes intolerant of study medication, alterations in the dose of concomitant medications should be considered prior to dose adjustment of study medication. Serum potassium will be determined at 48 hours after initiation of treatment, at 1 and 5 weeks, at all other scheduled study visits, and within one week following any dose change.

Study visits will occur at screening, baseline (randomization), 1 and 4 weeks, 3 months, and every 3 months thereafter until the study is terminated. Medical history, cardiac enzymes, Killip class, time to reperfusion (if applicable), documentation of AMI and of HF, determination of LVEF, and a serum pregnancy test for women of childbearing potential will be done at screening. A physical examination and 12-lead ECG will be done at screening and at the final visit (cessation of study drug). Hematology and biochemistry evaluations and urinalysis for safety will be done at screening, Week 4, Months 3 and 6, and every 6 months thereafter until the study is terminated. An additional blood sample for DNA analysis will be collected during screening. Vital signs (seated heart rate and BP), New York Heart Association (NYHA) functional class, adverse events, and selected

concurrent medications will be recorded at every visit. Quality of Life assessments will be completed during screening, at Week 4, Months 3, 6, and 12, and at the final visit. All randomized patients will be followed for endpoints (see below) every 3 months until the study is terminated.

The primary endpoint is all cause mortality. The trial is powered to detect an 18.5% reduction in all cause mortality, and requires 1,012 deaths before terminating the study. Secondary endpoints include:

1. cardiovascular mortality;
2. sudden cardiac death;
3. death due to progressive HF;
4. all cause hospitalizations;
5. cardiovascular hospitalizations;
6. HF hospitalizations;
7. all cause mortality plus all cause hospitalizations;
8. cardiovascular mortality plus cardiovascular hospitalizations;
9. cardiovascular mortality plus HF hospitalizations;
10. new diagnosis of atrial fibrillation;
11. hospitalization for recurrent non-fatal AMI and fatal AMI;
12. hospitalization for stroke; and
13. quality of life.

Safety will be assessed by adverse events, clinical laboratory values, physical examination, vital signs, and electrocardiogram.

Based on the results of the RALES study, it is hypothesized that eplerenone 25 to 50 mg QD will reduce mortality and morbidity in patients with HF post-AMI. Dose selection in this study is based on the results of the Phase II eplerenone HF and hypertension trials, in which eplerenone 25 to 50 mg QD increased plasma renin and aldosterone levels, lowered plasma BNP levels, but was neither diuretic nor hemodynamic. This study is designed to evaluate the effect of extended eplerenone

treatment in patients with HF after AMI.

It is obligatory that the Investigator become familiar with all sections of the Eplerenone Investigational Brochure prior to initiation of the study.

2.0 OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to compare the effect of eplerenone plus standard therapy versus placebo plus standard therapy on the rate of all cause mortality in patients with HF after AMI.

2.2 Secondary Objectives

The secondary objectives of this study are to compare the two treatment groups for:

1. cardiovascular mortality;
2. sudden cardiac death;
3. death due to progressive HF;
4. all cause hospitalizations;
5. cardiovascular hospitalizations;
6. HF hospitalizations;
7. all cause mortality plus all cause hospitalizations;
8. cardiovascular mortality plus cardiovascular hospitalizations;
9. cardiovascular mortality plus HF hospitalizations;
10. new diagnosis of atrial fibrillation;
11. hospitalization for recurrent non-fatal AMI and fatal AMI;
12. hospitalization for stroke; and
13. quality of life.

All endpoint events will be adjudicated by a blinded Critical Events Committee (see Section 5.5.b).

3.0 MATERIALS AND METHODS

3.1 Study Design and Procedures

This multicenter, randomized, double-blind, placebo-controlled, two-arm, parallel group trial will continue until 1,012 deaths occur, which is estimated to require

approximately 6,200 randomized patients followed for an average of approximately 2.5 years.

Eligible patients can be identified for inclusion at any time following emergency room evaluation and presumptive diagnosis of AMI with HF. Patients eligible for this study must have:

- AMI (the index event) documented by:
  - abnormal cardiac enzymes (creatine phosphokinase [CPK] >2x upper limit of normal [ULN] and/or CPK-MB [CPK MB isozyme band] >10% of total CPK); and
  - evolving electrocardiogram (ECG) diagnostic of MI (i.e., progressive changes in the ST segment and T wave compatible with AMI with or without the presence of pathological Q waves)
- LV dysfunction, documented by LV ejection fraction (LVEF) ≤40% by echocardiogram, radionuclide angiography, or LV angiography determined following the index AMI and before randomization.
- HF documented by at least one of the following:
  - pulmonary edema (bilateral posttussive crackles extending at least one-third of the way up the lung fields in the absence of significant chronic pulmonary disease); OR
  - chest x-ray showing pulmonary venous congestion with interstitial or alveolar edema; OR
  - auscultatory evidence of a third heart sound (S_3) with persistent tachycardia (>100 beats per minute).

Patients will receive standard therapy which may include ACE-I, diuretics, nitrates, and β-blockers, and may have received anticoagulants and antiplatelet agents, and may have received thrombolytics or emergency angioplasty.

Eligible patients may be identified for inclusion at any time following emergency room evaluation and presumptive diagnosis of AMI with HF. Patients who qualify

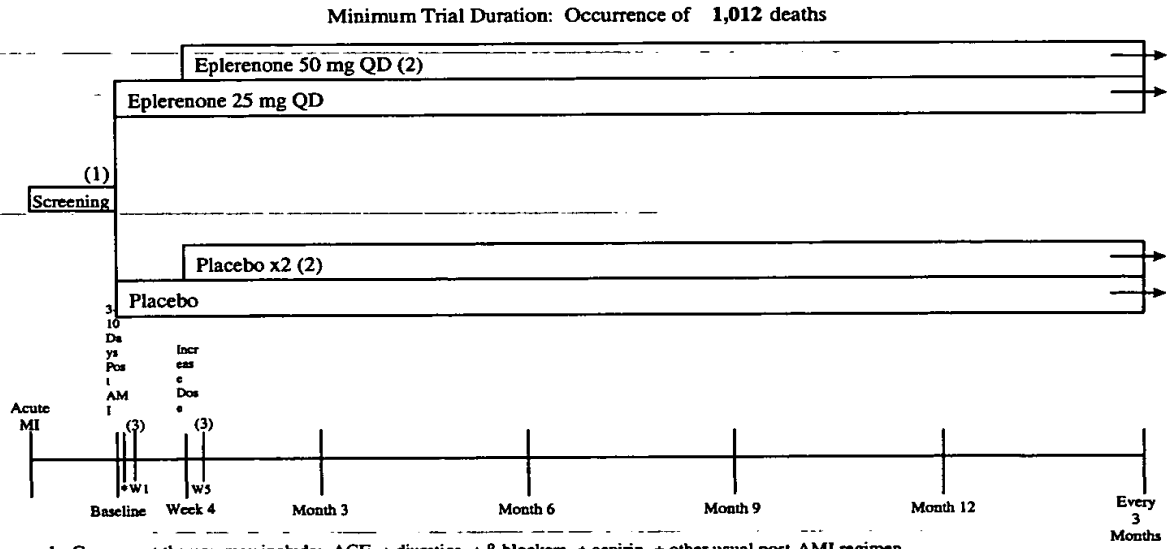
for the study will be randomized between 3 (>48 hours) and 10 days post-AMI if their clinical status is stable, e.g., no vasopressors or inotropes, intra-aortic balloon pump (IABP), hypotension (SBP<90 mmHg), or recurrent chest pain likely to lead to acute coronary arteriography. Patients with implanted cardiac defibrillators are excluded. Randomization should preferably occur prior to hospital discharge.

Patients will be randomized to receive eplerenone 25 mg QD (once daily) or placebo. At four weeks, the dose of study drug will be increased to 50 mg QD (two tablets) if serum potassium <5.0 mEq/L. If at any time during the study the serum potassium is >5.5 mEq/L but <6.0 mEq/L, the dose of study drug will be reduced to the next lower dose level, i.e., 50 mg QD to 25 mg QD (one tablet), 25 mg QD to 25 mg QOD (every other day), or 25 mg QOD to temporarily withheld (see Section 3.6 for detailed dosing instructions). If at any time during the study the serum potassium is ≥6.0 mEq/L, study medication is to be temporarily withheld, and may be restarted at 25 mg QOD when the serum potassium level is <5.5 mEq/L, and increased according to the schema presented in Section 3.6, Table 19. If at any time during the study the serum potassium is persistently ≥6.0 mEq/L, study medication is to be permanently discontinued. The potassium level may be repeated if the potassium increase is thought to be spurious (i.e., due to hemolysis or recent dosing with a potassium supplement). If the patient becomes intolerant of study medication, alterations in the dose of concomitant medications should be considered prior to dose adjustment of study medication. Serum potassium will be determined at 48 hours after initiation of treatment, at 1, 4 and 5 weeks, at all other scheduled visits, and within one week following any dose change. Dosing adjustments are to be made based on the most recent potassium level, as described in Section 3.6.

Study visits will occur at screening, baseline (randomization), 1 and 4 weeks, 3 months, and every 3 months thereafter until the study is terminated. At screening, medical history, cardiac enzymes, Killip class, time to reperfusion (if applicable), documentation of AMI and of HF, determination of LVEF, and a serum pregnancy test for women of childbearing potential will be done. A physical examination will

be done at screening and at the final visit (cessation of study drug). Hematology and biochemistry evaluations and urinalysis for safety will be done at screening, Week 4, Months 3 and 6, and every 6 months thereafter until the study is terminated. An additional blood sample for DNA analysis will be collected during screening. Vital signs (seated heart rate and BP), New York Heart Association (NYHA) functional class, adverse events, and selected concurrent medications will be recorded at every visit. Quality of Life (QOL) assessments will be completed during screening, at Week 4, Months 3, 6, and 12, and at the final visit. All randomized patients will be followed for all study endpoints (see Section 2.0) every 3 months until the study is terminated.

Study Schematic



1 Concurrent therapy may include: ACE, ± diuretics, ± ß-blockers, ± aspirin, ± other usual post-AMI regimen.

2 Increase dose if current dose is tolerated and serum potassium is <5.0 mEq/L. See Section 3.6 and Table 1 for complete instructions regarding study medication dose adjustments.

3 Serum potassium must be determined at 48 hours, Week 1, Week 5 and within 1 week following any study medication dose change. Dose adjustments are to be made based on the most recent potassium level. If at any time during the study, serum potassium is >5.5 mEq/L but <6.0 mEq/L, reduce the dose of study medication to the next lower dose. If at any time during the study, serum potassium is ≥6.0 mEq/L, temporarily withhold study medication. If study medication is temporarily withheld, it may be restarted at one tablet QOD when serum potassium is <5.5 mEq/L and titrated as directed in Section 3.6, Table 1. If at any time during the study the serum potassium is persistently ≥6.0 mEq/L, permanently discontinue study medication.

* Serum potassium determination at 48 hours after initiation of dosing.

3.2 Study P pulati n

3.2.a Patient Enrollment

A sufficient number of patients will be enrolled to ensure that a total of 1,012 deaths will occur over the course of the study (see Section 5.0).

3.2.b Criteria for Inclusion

1. The patient has had an AMI, as documented by:
   a. cardiac enzyme rise (total CPK >2xULN and/or CPK-MB >10% of total CPK), AND
   b. evolving ECG diagnostic of MI (i.e., progressive changes in the ST segment and T wave compatible with AMI with or without the presence of pathological Q waves)
2. The patient has LV dysfunction, as documented by LVEF ≤40% by echocardiogram, radionuclide angiography, or LV angiography determined following the index AMI and before randomization.
3. The patient has clinical evidence of HF, demonstrated by at least one of the following:
   a. pulmonary edema (bilateral posttussive crackles extending at least one-third of the way up the lung fields in the absence of significant chronic pulmonary disease); OR
   b. chest x-ray showing pulmonary venous congestion with interstitial or alveolar edema; OR
   c. auscultatory evidence of a third heart sound (S_3) with persistent tachycardia (>100 beats per minute).

   Clinical evidence of HF post AMI can be transient, occurring at any time from the onset of the index AMI prior to randomization. Evidence of HF does not necessarily need to be present at the time of randomization.
4. Stable clinical status at the time of randomization 3 (>48 hours) to 10 days following the AMI. Stable clinical status excludes the use of vasopressors and inotropes. Stable status also excludes the use of an IABP,

hypotension (SBP<90 mmHg), and recurrent chest pain likely to lead to acute coronary arteriography.

5. The patient is a male or nonpregnant female ≥21 years of age.
6. If the patient is a female, she is post-menopausal, or if of childbearing potential, she is using adequate contraception (hormonal, e.g., oral contraceptives or hormonal implants, or barrier method, e.g., diaphragm, IUD, etc.), or is surgically sterile, and is not lactating. Abstinence is not an acceptable form of contraception.
7. If the patient is a female of childbearing potential, she has had a negative serum pregnancy test within 72 hours prior to the first scheduled dose of double-blind study drug.
8. The patient has no clinically significant abnormal clinical laboratory values which in the Investigator's opinion precludes the patient from safely participating in this study.
9. The patient is willing and able to participate for the duration of the study.
10. The patient has provided written informed consent prior to any test or procedure being performed, or medication being changed, for this study.

3.2.c Criteria for Exclusion

1. The patient has HF of primary valvular or congenital etiology.
2. The patient has current evidence of clinical instability (e.g., arrhythmias other than atrial fibrillation, cardiogenic shock, etc.).
3. The patient has post-infarct angina likely to lead to acute coronary arteriography.
4. A coronary artery bypass graft (CABG) is planned for the index AMI.
5. The patient has an implanted cardiac defibrillator (ICD).
6. The patient has uncontrolled hypotension (SBP<90 mmHg).
7. The patient requires the use of potassium-sparing diuretics or spironolactone.
8. The patient has a serum creatinine level >2.5 mg/dL during the screening period.

9. The patient has a serum potassium level >5.0 mEq/L during the screening period.
10. The patient has a planned cardiac transplantation.
11. The patient has current evidence of alcohol or drug abuse problems, which in the Investigator's opinion, precludes study participation.
12. The patient has any condition which, in the Investigator's opinion, makes participation in this study not in the best interest of the patient.
13. The patient has known hypersensitivity to eplerenone or spironolactone.
14. The patient has a severe organic disorder or has had surgery or disease of the gastrointestinal tract which, in the opinion of the Investigator, may interfere with the absorption, pharmacokinetics, or elimination of the study medication.
15. The patient has chronic psychoses or behavioral conditions that in the opinion of the Investigator would limit the ability of the patient to comply with the requirements of this study.
16. The patient has a comorbid condition that would be expected to result in death during the next three years (e.g., terminal cancer, AIDS, etc.) including patients receiving immunosuppressive or antineoplastic therapy.
17. The patient has received any investigational medication or investigational device within 30 days prior to the first dose of study medication or is actively participating in any investigational drug or device study, or is scheduled to receive an investigational drug other than eplerenone or be treated with an investigational device during the course of this study.
18. The patient has been previously admitted to the study.

3.3 Randomization Procedures

Patients will be assigned at each site to a double-blind treatment arm in the order in which they meet criteria for randomization (see Sections 3.2.b and 3.2.c). They will receive their allocated treatment according to a computer-generated randomization schedule prepared at Searle prior to the start of the study.

3.3.a Generation of Randomization Code

The Searle clinical database administrator will generate the patient randomization schedule using Searle's standard randomization program. The Searle statistician will generate the randomization schedule for medication kit identification numbers, separate from the patient randomization schedule. The randomizations will be provided to a drug packaging contractor and to the Interactive Voice Response System (IVRS) center for drug assignments. The Searle statistician will not have access to the randomization codes after patient recruitment begins. The Searle clinical database administrator will keep both the patient randomization schedule and the medication identification schedule in a locked file for the duration of the study. A sealed copy of the patient randomization schedule will be provided to the U.S. Food and Drug Administration (FDA) prior to the start of the study. The randomization schedule will also be made available to the unblinded statistical group performing the statistical analyses for the Data Safety Monitoring Board (DSMB). A designated individual in the Searle pharmacy will have access to the medication kit identification randomization for drug packaging purposes only. No other Searle personnel will have access to the randomization schedules until the study is completed.

In general, no study site personnel will have access to the randomization codes. However, if it is medically necessary to unblind a particular patient, a sealed treatment assignment is provided with each study medication package (see Section 3.5).

3.3.b Interactive Voice Randomization System (IVRS)

A 24-hour IVRS will be used to assign patient numbers and blinded study drug to patients, track inventories of study drug at sites, and track recruitment and progress of patients.

Site personnel will call the IVRS center to randomize each patient. The Investigator will provide the IVRS center with various identifiers for each patient and confirm

that inclusion/exclusion criteria have been met. The IVRS center will assign the patient to a treatment according to a patient randomization schedule described above. The IVRS system will then select a blinded medication identification number appropriate to the patient's treatment assignment from those available at the study site.

Following randomization, sites are to call the IVRS center whenever study drug is dispensed. The IVRS center must also be notified if the patient withdraws from treatment or is unblinded. Furthermore, the IVRS center must be called to confirm receipt of study drug supplies.

Additional details of the IVRS system and procedures will be provided in a separate IVRS manual.

3.3.c Emergency Code Break

In the event of an emergency, the site will be provided a telephone number to call in order to request to have the study blind broken. The code may be broken if an emergency situation arises that in the Investigator's opinion requires knowledge of the code. In these cases, the Investigator should attempt to contact the Sponsor before breaking the code. The date and reason for the code break must be submitted on the appropriate CRF to the data coordinating center by the Investigator as soon as possible.

3.4 Description of Clinical Supplies

Searle will provide eplerenone 25 mg tablets and matching placebo for eplerenone 25 mg tablets, as follows:

Initial Phase Study Medication (Weeks 0 through 4)

Bottles of 40 tablets with child-resistant closure to be dispensed at baseline (Day 0).

Maintenance Phase Study Medication (After Week 4)

Bottles of 200 tablets with child-resistant closure to be dispensed for the remainder of the study.

Double-blind eplerenone or matching placebo study medication will be supplied in bottles pre-labeled with appropriate kit numbers for each treatment arm. Prior to dispensing, all study medication must be stored according to labeled storage conditions in a secure area with limited access. At home, the patient must keep the medication free from environmental extremes. When the study is completed or discontinued, all used and unused supplies of drug must be returned or disposed of, as directed by the Searle Monitor or monitors designated by Searle.

3.5 Labeling of Clinical Supplies

Two-part labels will be computer-generated for this blinded study. One part of the label, containing study and patient information will be attached to the container; the other part is a tear-off portion that contains the same information plus a sealed pouch containing the identity of the assigned treatment. This tear-off tab is to be removed at the time of dispensing, attached to the patient's appropriate CRF and retained in the Investigator's study file.

The following dosage instructions will appear on the label in the language(s) appropriate to the country in which the drug will be used:

Initial Phase Bottle

"Take one tablet every morning with water or as otherwise directed by your physician."

Maintenance Phase Bottle

"Take two tablets every morning with water or as otherwise directed by your physician."

See Section 3.3 and Section 3.0 for detailed information on randomization and the

blinding safeguards.

3.6 Study Drug Administration

Patients will receive eplerenone 25 mg QD or placebo (one tablet) for the first four weeks of treatment. At four weeks, the dose of study drug will be increased to 50 mg QD (two tablets) if serum potassium <5.0 mEq/L. If the serum potassium is ≥5.0 mEq/L at Week 4 but <5.0 mEq/L at Week 5, the dose of study drug will be increased to 50 mg QD (two tablets). In this case, serum potassium is to be checked at Week 6.

Table 20 summarizes mandated dosing changes for serum potassium levels. Serum potassium will be determined at 48 hours after initiation of treatment, at 1 and 5 weeks, and within one week following any dose change. If at any time during the study the serum potassium is >5.5 mEq/L, the dose of study drug will be reduced to the next lower dose level, i.e., 50 mg QD to 25 mg QD, 25 mg QD to 25 mg QOD, or 25 mg QOD to temporarily stopped. Study medication is to be restarted at 25 mg QOD when the serum potassium level is <5.5 mEq/L and increased according to the scheme presented in Table 20. The potassium level may be repeated if the potassium increase is thought to be spurious (i.e., due to hemolysis or recent dosing with a potassium supplement).

If the patient becomes intolerant of study medication, alterations in the dose of concomitant medications (e.g., potassium supplements, ACE-I, etc.) should be considered prior to dose adjustment of study medication. If at any time during the study the serum potassium level is ≥6.0 mEq/L, study medication is to be temporarily withheld. If serum potassium level is persistently ≥6.0 mEq/L, the patient is to discontinue study medication. If elevated potassium levels are observed <6.0 mEq/L, potassium supplements, if any, should be stopped and the patient should continue to receive study medication. If study medication is stopped, concurrent medications should be reviewed and the doses adjusted if possible according to good clinical practice.

Table 20.

Study Medication Dosing Adjustment for Serum Potassium Levels

If Serum Potassium Level Is:	And Current Dose Is:	Dose Change:	Number of Tablets:
<5.0 mEq/L	Withhold	Increase	1 tablet QOD
<5.0 mEq/L	1 tablet QOD	Increase	1 tablet QD
<5.0 mEq/L	1 tablet QD	Increase	2 tablets QD
<5.0 mEq/L	2 tablets QD	No change	2 tablets QD
≥5.0 and <5.5 mEq/L	Withhold	Increase	1 tablet QOD
≥5.0 and <5.5 mEq/L	1 tablet QOD	No change	1 tablet QOD
≥5.0 and <5.5 mEq/L	1 tablet QD	No change	1 tablet QD
≥5.0 and <5.5 mEq/L	2 tablets QD	No change	2 tablets QD
≥5.5 and <6.0 mEq/L	Withhold	No change	None
≥5.5 and <6.0 mEq/L	1 tablet QOD	Decrease	None
≥5.5 and <6.0 mEq/L	1 tablet QD	Decrease	1 tablet QOD
≥5.5 and <6.0 mEq/L	2 tablets QD	Decrease	1 tablet QD
≥6.0 mEq/L	Any dose	*	None

*If persistent elevation, discontinue medication. If single elevation, withhold dosing.

4.0 STUDY PLAN

Schedule of Observations and Procedures

Screening Period

The Screening Period is defined as the period after AMI and prior to randomization. This section describes the procedures that must be done during the Screening Period:

Written informed consent must be obtained for each patient prior to any study-related procedure or change in medication for the purpose of this study.

The inclusion/exclusion criteria will be reviewed and used to determine each patient's potential eligibility for the study.

The time to reperfusion following AMI will be recorded, if applicable.

4.2.a Medical History, Physical Examination, Vital Signs, and Electrocardiogram

Medical history will include specific questions to allow identification of underlying cardiac disease.

Physical examination will include measurement of body weight and height, and assessment of presence or absence of pulmonary rales, gallop sound (S_3) with persistent tachycardia, and peripheral edema.

Vital signs will include measurement of seated heart rate and BP by cuff (sphygmomanometer).

12-Lead ECG will be performed.

4.2.b Documentation of Acute Myocardial Infarction, Left Ventricular Dysfunction, and Heart Failure

Documentation of the index AMI includes:

1. Cardiac enzyme rise:
   - total CPK >2xULN and/or
   - CPK-MB >10% of total CPK, AND
2. ECG diagnostic of MI (i.e., progressive ST segment and T wave compatible with AMI with or without the presence of pathological Q waves)

LV dysfunction is to be documented by LVEF ≤40% by echocardiogram, radionuclide angiography, or LV angiography determined following the index AMI and before randomization.

Documentation of HF includes at least one of the following:

1. Pulmonary edema (bilateral posttussive crackles extending at least one-third of the way up the lung fields in the absence of significant chronic pulmonary disease);
2. Chest x-ray showing pulmonary venous congestion with interstitial or alveolar edema; OR
3. Auscultatory evidence of a third heart sound (S_3) with persistent tachycardia (>100 beats per minute).

Clinical evidence of HF post AMI can be transient, occurring at any time from the onset of the index AMI prior to randomization. Evidence of HF does not necessarily need to be present at the time of randomization.

4.2.c Clinical Laboratory Tests

Clinical safety laboratory tests will be performed for the following parameters. If possible, blood collection should occur in the morning:

Hematology

WBC with differential	Platelet count (estimate not acceptable)
RBC	
Hemoglobin	
Hematocrit	

Biochemistry

Sodium	Glucose
Potassium	Alkaline phosphatase
Chloride	Gamma glutamyl transferase (γ-GT)
Calcium	SGOT (AST)
Magnesium	SGPT (ALT)
Phosphate (inorganic)	LDH
BUN (urea)	Creatine phosphokinase (CPK)
Creatinine	CPK-MB (screening only)

Total protein	Total cholesterol
Total bilirubin (direct and indirect)	LDL Cholesterol
Albumin	HDL Cholesterol
Uric acid	Triglycerides

Serum Pregnancy Test for women of childbearing potential.

Urinalysis

pH	Protein
Specific gravity	Glucose
Ketones	Blood

The Investigator will review all laboratory test results and initial each laboratory report. Any abnormal pretreatment values that require clinical intervention or that the Investigator considers clinically significant will exclude the patient from study participation. All laboratory tests will be performed by the designated central laboratory or local laboratory as appropriate. For sites using the central laboratory, instructions and materials for collecting and shipping of samples will be provided to each study site by the central laboratory.

4.2.d Killip Class

The patient's current Killip class will be determined as follows:

Class I: absence of rales and a third heart sound

Class II: rales up to 50% of each lung field or the presence of the third heart sound

Class III: rales in more than 50% of each lung field

Class IV: cardiogenic shock (resulting from decline in cardiac output secondary to serious heart disease, usually AMI)

4.2.e New York Heart Association (NYHA) Functional Classification

The patient's current NYHA functional classification will be determined as follows:

Class I:	no symptoms with ordinary physical activity
Class II:	symptoms with ordinary physical activity but not at rest
Class III:	symptoms with less than ordinary physical activity but not at rest
Class IV:	symptoms present at rest

4.2.f Quality of Life Assessments

Patients enrolled in the trial in the United States, United Kingdom, Spain, Canada, Brazil, Germany, Belgium, France, The Netherlands and Argentina will complete the following QOL assessments during the Screening period and before the first dose of study medication.

1. **The Kansas City Cardiomyopathy Questionnaire (KCCQ):** This disease-specific instrument for patients with HF has been extensively tested and validated to ensure the accuracy of its assessments. It quantifies the full range of health status as impacted upon by the syndrome of HF. The 23-item KCCQ specifically quantifies symptoms (their frequency, severity, and change over time), function (physical and social), and quality of life. Disease-specific measures have repeatedly been demonstrated to be more sensitive to clinical change than generic health status measures, and the KCCQ should provide a robust assessment of eplerenone's impact on the health status of patients. (Spertus JA, et al. *Am J Cardiol* 1994;74:1240-1244.)

2. **The Short Form - 12 Health Survey (SF-12):** The SF-12 is a generic 12-item questionnaire that can generate an overall summary score of physical and mental health. (Jenkinson C et al. *J Public Health Med* 1997;19:179-186.) Unlike the KCCQ, it is not specific for HF and will capture the limitations in health posed by other comorbid conditions. Nevertheless, population norms for this

instrument are available and will allow benchmarking of the patient population against other studies (including those for the treatment of different diseases).

3. **The EuroQoL Health Rating Scale:** The EuroQoL quantifies three levels of function in five different generic domains. (Kind P. The EuroQoL instrument: An index of health-related quality of life. In: Spilker B, ed. Quality of life and pharmacoeconomics in clinical trials. 2nd edition. Philadelphia: Lippincott-Raven; 1996:191-201.) With the inclusion of a "feeling thermometer": it is composed of six questions and can efficiently synthesize the range of health status into a single number (utility) that will be used for the planned economic analyses of this trial. (Torrence GW. *J Health Econ* 1986;5:1-30.)

4. **The Medical Outcomes Study Depression Scale (MOS-D):** The MOS-D was developed for use in the National Study of Medical Outcomes as a screening device for depressive disorders in a medical patient population. (Burnham MA et al. *Med Care* 1988;26:775-789) It consists of eight items that were incorporated items from the Diagnostic Interview Schedule (DIS) and the Center for Epidemiological Studies Depression Scale (CES-D), which do not use somatic indicators of depression, a potential confounding marker of depression among patients with HF. The MOD-D has excellent sensitivity (93% (95% CI 86-97)) and good specificity (72% (95% CI 68-76)). The MOS-D will be used both for discriminating clinically relevant subsets at baseline (i.e., depressed vs. nondepressed patients) for the secondary and tertiary objectives of defining baseline predictors of greater benefit (both mortality and health status outcomes) from eplerenone and as an assessment of the responsiveness of depression to treatment with eplerenone - an additional secondary objective of this study.

5. **Brief Symptom Inventory-Anxiety (BSI-A):** The BSI-A is a shorter alternative to the Symptom Checklist 90 - Revised. It is comprised of six items that measure anxiety. (Derogatis LR, Melisaratos N. *Psychol Med* 1983;13:595-605) Like the MOS-D it has the advantage of not using physical indicators of

emotional states, which can overestimate the level of mood states in patients with cardiovascular disease. The BSI-A has high internal consistency (Cronbach's alpha = 0.85) and well established construct, convergent, discriminant, and predictive validity. Given the emerging literature on the association between anxiety and cardiovascular disease, baseline anxiety will be used to evaluate differential effects of eplerenone therapy on health status and mortality outcomes (secondary and tertiary objectives of this study). The brevity and excellent psychometric properties of the BSI-A make this measure an exceptional one for broaching this new area of investigation.

4.2.g Blood Sample for DNA Analysis

A 30 mL blood sample for DNA analysis will be obtained. From this sample, 20 mL will be put into tubes containing EDTA and 10 mL will be put into tubes containing sodium citrate. The tubes will be placed at 4°C until courier collection, and shipped immediately by courier to the designated laboratory.

4.2.h Admission of Patients

After screening, eligible patients are to be given study numbers in sequence. In addition, they are to be identified by first, middle, and last initials. If the patient has no middle initial, a dash is to be used.

4.3 Treatment Period

4.3.a Concurrent Medications

Potassium-sparing diuretics and spironolactone are prohibited for the duration of this study. There are no other restrictions on concomitant medications in this trial. Any medication is permitted if, in the opinion of the Investigator, it is necessary. Patients should avoid any additional medication (including over-the-counter [OTC] drugs) without prior approval of the Investigator.

For all concomitant medications, the start and stop dates, as well as the reason for use, must be recorded on the appropriate CRF. For selected concomitant

medications (including ACE-I, AII antagonists, antiarrhythmics, anticoagulants, antiplatelet agents, ß-blockers, calcium channel blockers, digoxin, diuretics, magnesium supplements, potassium supplements, and α-blockers), the dose, as well as start and stop dates must be recorded on the appropriate CRF. All changes in these selected concomitant medications must be recorded on the appropriate CRF.

4.3.b Visit 1 Baseline and Randomization (Day 0)

Randomization must occur between 3 days (>48 hours) to 10 days after the onset of an AMI (earliest onset of symptoms), preferably prior to discharge from the hospital.

At Visit 1 (Day 0) patients will be assessed for eligibility for the Treatment Period. Visit 1 assessments include:

1. Seated heart rate and seated cuff BP.
2. Determination of NYHA functional classification (see Section 4.2.e).
3. Recording of concurrent medications.
4. Inclusion/exclusion criteria determination (see Sections 3.2.b and 3.2.c).

Each eligible patient will be assigned the next available four-digit patient number and will receive the treatment assigned to that number by a computer-generated randomization schedule prepared at Searle prior to the start of the study.

Patients will receive a four-week supply of double-blind study medication and, again, be instructed to take one tablet every morning with water, or as otherwise directed by the Investigator.

4.3.c Safety Laboratory Assessments (48 hours Post-Randomization)

Serum potassium level will be determined at 48 hours after the initiation of dosing. If possible, blood for this measure should be drawn in the morning.

If at any time during the study, the serum potassium level is >5.5 mEq/L but <6.0 mEq/L, the dose of study medication will be reduced to the next lower dose, e.g., 25 mg QD to 25 mg QOD, 25 mg QOD to temporarily withheld. Serum potassium level is to be determined within one week after each dose adjustment. Dose adjustments are to be made based on the most recent serum potassium level. If at any time during the study the serum potassium level is ≥6.0 mEq/L, study medication is to be temporarily withheld. If study medication is temporarily withheld, it may be restarted at one tablet QOD when the serum potassium is <5.5 mEq/L and titrated as directed in Section 3.6, Table 20. See Section 3.6 for detailed dosing instructions and for instructions for restarting study medication. If at any time during the study the serum potassium level is persistently ≥6.0 mEq/L, study medication is to be permanently discontinued.

4.3.d Visit 2 (1 Week ± 3 Days Post-Randomization)

The following procedures will be performed after one week of treatment:

1. Seated heart rate and seated cuff BP.
2. Blood draw for serum potassium level. If at all possible, blood should be collected in the morning.
3. Determination of NYHA functional classification (see Section 4.2.e).
4. Assessment of endpoints (see Section 2.0).
5. Recording of any new concurrent medications and any dose changes for selected medications (see Section 4.3.a).
6. Recording of any adverse events.
7. Counting of returned medications and recording of compliance.

If at any time during the study, the serum potassium level is >5.5 mEq/L but <6.0 mEq/L, the dose of study medication will be reduced to the next lower dose, e.g., 50 mg QD (two tablets) to 25 mg QD (one tablet), 25 mg QD to 25 mg QOD, 25 mg QOD to temporarily withheld. Serum potassium level is to be determined within one week after each dose adjustment. Dose adjustments are to be made based on the most recent serum potassium level. If at any time during the study the

serum potassium level is ≥6.0 mEq/L, study medication is to be temporarily withheld. If study medication is temporarily withheld, it may be restarted at one tablet QOD when the serum potassium is <5.5 mEq/L and titrated as directed in Section 3.6, Table 20. See Section 3.6 for detailed dosing instructions and for instructions for restarting study medication. If at any time during the study the serum potassium level is persistently ≥6.0 mEq/L, study medication is to be permanently discontinued.

4.3.e Visit 3 (4 Weeks ± 3 Days Post-Randomization)

The following procedures will be performed after four weeks of treatment:

1. Seated heart rate and seated cuff BP.
2. Clinical safety laboratory blood draw including potassium (see Section 4.2.c).
3. Clinical safety urine sample for urinalysis (see Section 4.2.c).
4. Determination of NYHA functional classification (see Section 4.2.e).
5. Assessment of endpoints (see Section 2.0).
6. QOL assessments (see Section 4.2.f).
7. Recording of any new concurrent medications and any dose changes for selected medications (see Section 4.3.a).
8. Recording of any adverse events.
9. Counting of returned medications and recording of compliance.
10. Dispensing of a three-month supply of study medication with medication diary card.

At this visit, the patient will be instructed to continue on 25 mg QD eplerenone/placebo. If the serum potassium level measured at this visit is <5.0 mEq/L, the site will contact the patient and instruct the patient to increase to dose to two tablets per day. If the serum potassium is ≥5.0 mEq/L at Week 4 but <5.0 mEq/L at Week 5, the dose of study drug will be increased to 50 mg QD (two tablets). In this case, serum potassium is to be checked at Week 6. If at any time during the study, the serum potassium level is >5.5 mEq/L but <6.0 mEq/L, the

dose of study medication will be reduced to the next lower dose, e.g., 50 mg QD (two tablets) to 25 mg QD (one tablet), 25 mg QD to 25 mg QOD, 25 mg QOD to temporarily withheld. Serum potassium level is to be determined within one week after each dose adjustment. Dose adjustments are to be made based on the most recent serum potassium level. If at any time during the study the serum potassium level is ≥6.0 mEq/L, study medication is to be temporarily withheld. If study medication is temporarily withheld, it may be restarted at one tablet QOD when the serum potassium is <5.5 mEq/L and titrated as directed in Section 3.6, Table 20. See Section 3.6 for detailed dosing instructions and for instructions for restarting study medication. If at any time during the study the serum potassium level is persistently ≥6.0 mEq/L, study medication is to be permanently discontinued.

4.3.f Safety Laboratory Assessment (5 Weeks Post-Randomization)

Serum potassium level is to be determined at Week 5 for all patients. For patients whose dose level was not increased at Week 4, the dose of study medication may be increased to two tablets QD if the serum potassium is <5.0 mEq/L. If the dose is increased at this visit, the serum potassium level is to be determined within one week. If possible, blood for this measure should be drawn in the morning.

If at any time during the study, the serum potassium level is >5.5 mEq/L but <6.0 mEq/L, the dose of study medication will be reduced to the next lower dose, e.g., 50 mg QD (two tablets) to 25 mg QD (one tablet), 25 mg QD to 25 mg QOD, 25 mg QOD to temporarily withheld. Serum potassium level is to be determined within one week after each dose adjustment. Dose adjustments are to be made based on the most recent serum potassium level. If at any time during the study the serum potassium level is ≥6.0 mEq/L, study medication is to be temporarily withheld. If study medication is temporarily withheld, it may be restarted at one tablet QOD when the serum potassium is <5.5 mEq/L and titrated as directed in Section 3.6, Table 20. See Section 3.6 for detailed dosing instructions and for instructions for restarting study medication. If at any time during the study the

serum potassium level is persistently ≥6.0 mEq/L, study medication is to be permanently discontinued.

4.3.g Visits 4 onward (3 Months ± 10 Days Post-Randomization and Every 3 Months Thereafter)

The following procedures will be performed after three months of treatment and every three months thereafter:

1. Seated heart rate and seated cuff BP.
2. Clinical safety laboratory blood draw including potassium and clinical safety urine sample for urinalysis (see Section 4.2.c) will be performed at Months 3, 6, 12, 18, 24, and every six months thereafter as long as the study continues. A blood draw for serum potassium only will be performed at Months 9, 15, 21, and every three months thereafter as long as the study continues.
3. Determination of NYHA functional classification (see Section 4.2.e).
4. Assessment of endpoints (see Section 2.0).
5. QOL assessments will be performed at Months 3, 6, and 12 (see Section 4.2.f).
6. Recording of any new concurrent medications and any dose changes for selected medications (see Section 4.3.a).
7. Recording of any adverse events.
8. Counting of returned medications and recording of compliance.
9. Dispensing of a three-month supply of study medication with medication diary card.

If at any time during the study, the serum potassium level is >5.5 mEq/L but <6.0 mEq/L, the dose of study medication will be reduced to the next lower dose, e.g., 50 mg QD (two tablets) to 25 mg QD (one tablet), 25 mg QD to 25 mg QOD, 25 mg QOD to temporarily withheld. Serum potassium level is to be determined within one week after each dose adjustment. Dose adjustments are to be made based on the most recent serum potassium level. If at any time during the study the

serum potassium level is ≥6.0 mEq/L, study medication is to be temporarily withheld. If study medication is temporarily withheld, it may be restarted at one tablet QOD when the serum potassium is <5.5 mEq/L and titrated as directed in Section 3.6, Table 20. See Section 3.6 for detailed dosing instructions and for instructions for restarting study medication. If at any time during the study the serum potassium level is persistently ≥6.0 mEq/L, study medication is to be permanently discontinued.

4.3.h Final Visit or Early Cessation of Study Medication

The following procedures will be performed at the final visit:

1. Seated heart rate and seated cuff BP.
2. Physical examination.
3. 12-lead electrocardiogram.
4. Clinical safety laboratory blood draw including potassium (see Section 4.2.c).
5. Clinical safety urine sample for urinalysis (see Section 4.2.c).
6. Determination of NYHA functional classification (see Section 4.2.e).
7. Assessment of endpoints (see Section 2.0).
8. QOL assessments (see Section 4.2.f).
9. Recording of any new concurrent medications and any dose changes for selected medications (see Section 4.3.a).
10. Recording of any adverse events.
11. Counting of returned medications and recording of compliance.

Any abnormal findings at the final assessment should be followed by the Investigator until satisfactorily resolved.

4.4 Permanent Discontinuation of Study Medication Prior to Study Termination

If for any reason a patient permanently discontinues study medication before the study is terminated, the reason for such discontinuation must be entered on the Permanent Cessation of Study Drug Form. The patient's permanent discontinuation

of study medication must also be registered with the IVRS center by the Coordinator or Investigator. The patient must undergo procedures described in Section 4.3.h, Final Visit or Early Cessation of Study Medication, and appropriate CRFs must be completed. All data for the patient prior to cessation of study medication will be made available to G.D. Searle & Co., and the patient will continue to be followed every three months by telephone for endpoints (see Section 2.0) until the end of the study.

A patient may permanently discontinue study medication for any of the following:

1. Inability to tolerate study medication.
2. Persistent serum potassium level ≥6.0 mEq/L when the patient is receiving the lowest dose (1 tablet QOD).
3. Pregnancy.
4. Administrative reasons.
5. Any other reason which in the opinion of the Investigator is to protect the best interest of the patient.
6. Request of the patient to withdraw. The patient has the right to withdraw at any time for any reason.
7. Treatment with spironolactone.

It is understood by all that excessive stopping of study medication can render the study uninterpretable; therefore, unnecessary medication stoppage should be avoided. Clear description of trial procedures to patients and their understanding of the Informed Consent Form is not only mandatory, but crucial to limiting unnecessary medication stoppage.

5.0 STATISTICS

5.1 Justification of Sample Size

5.1.a Sample Size and Mortality Assumptions

Randomized patients will be followed until 1,012 deaths have occurred. This number will provide over 90% power to detect an 18.5% reduction in the rate of

death compared to the placebo group. When the first-year placebo mortality rate in the placebo group is 15% or greater and up to 6,200 patients are enrolled over an 18-month period, the target number of 1,012 deaths should occur within the first 30 months of the trial (18 month enrollment plus 12 months follow-up after the last patient is enrolled).

In order to estimate the required number of patients and duration of follow-up to achieve this number of deaths, data from three trials were considered. In these trials, the one-year mortality rate for the groups receiving active treatment ranged from 12% to 23%. In the AIRE trial, conducted in several European countries, and the TRACE trial, conducted in Denmark, the first-year mortality rates in the active (ACE-I) arms were 16% and 23%, respectively. (Køber L et al. *N Engl J Med* 1995;333:1670-1676) In the AIRE trial, among all patients randomized who were not receiving digoxin, the first-year rate was about 12% (about 17% for patients receiving digoxin). In the GISSI-3 trial, of patient randomized to lisinopril who were alive at Day 3 with Killip class >I, the first-year mortality was 22%; for these patients ACE-I treatment was stopped at 42 days, but more than 50% of them continued treatment long-term. The table below gives the required number of patients and duration of follow-up based on three different estimates for the one-year placebo mortality rate (12%, 15%, and 18%). These results were obtained using software described by Shih, 1995. (Shih JH. *Controlled Clinical Trials* 1995;16:395-407)

The calculations assume a decreasing hazard of mortality, similar to that seen in the active treatment arm of the AIRE trial. Similar results were obtained by using results from the TRACE trial. The active rather than placebo arms of these ACE-I trials were used because the current trial will allow background medication including ACE-I for all patients. It is also assumed that the hazard ratio between the two treatment arms is constant over time (proportional hazards) and that a greater rate of recruitment will occur in the final 12 months of the enrollment period than in the initial six months.

Table 21

Number of Patients to be Enrolled Under Various Mortality Assumptions

Reduction in Hazard of Death (%)	Target Number of Deaths	1st Year Placebo Mortality Rate (%)	Number of Patients To Be Enrolled (to nearest 100) 12 Months' Follow-up*	15 Months' Follow-up*	18 Months' Follow-up*	21 Months' Follow-up*	24 M nths' Follow-up*
15.0	1,598	12	11,600	10,800	10,100	9,400	8,900
		15	9,300	8,700	8,100	7,600	7,200
		18	7,800	7,300	6,800	6,400	6,000
18.5	1,012	12	7,500	6,900	6,500	6,100	5,700
		15	6,000	5,600	5,200	4,900	4,600
		18	5,000	4,700	4,400	4,100	3,900
22.0	687	12	5,200	4,800	4,500	4,200	4,000
		15	4,200	3,900	3,600	3,400	3,200
		18	3,500	3,200	3,000	2,800	2,700

*Follow-up after 18 months of enrollment

The mortality reduction over the course of the study was 27% in the AIRE trial and 22% in TRACE. Over the course of the current trial, a smaller treatment effect is expected than in the earlier trials, because the active treatment arms in the earlier trials are expected to be more similar to the placebo arm in this trial. Therefore, reductions in first-year mortality of 15%, 18.5% and 22% were considered in the sample size scenarios. Note that in the recent RALES trial (17), the active agent, with the same mechanism of action as eplerenone, achieved over a 30% reduction in mortality compared to placebo, albeit in a different patient population. In the RALES trial, most patients in each treatment arm received standard therapy in addition to their study medication.

5.1.b Loss to follow-up

Sample size estimates have not been adjusted for loss-to-follow-up, because study management procedures are expected to keep the loss below 1%.

5.2 Baseline and Demographic Analysis

Comparability of treatment groups with respect to baseline and demographic factors will be examined using t-tests for continuous variables and chi-square tests for categorical variables.

5.3 Efficacy Analysis

5.3.a Primary and Secondary Endpoints

All statistical analyses of the primary and secondary endpoints will follow the intention-to-treat principle. Data from randomized patients will be analyzed according to the patients' original treatment assignment, and all patients will be followed for mortality and other major endpoints for the duration of the study, regardless of compliance with taking study medication.

For each endpoint, the time-to-event will be analyzed using the logrank test at the overall 0.05 level, accounting as needed for interim analyses. To be included in the statistical analysis, any adjudicatable endpoint event must be adjudicated by the Critical Events Committee (see Section 5.5.b). Kaplan-Meier curves will be used to summarize the various time-to-event distributions. Exploratory analyses of these endpoints using baseline characteristics as covariates may be performed using Cox proportional hazards regression to estimate relative hazard rates and 95% confidence intervals.

The logrank tests and Cox regression analyses will be stratified by geographic region. The regions will consist of the United States and Canada; Latin America; Eastern Europe; and Western Europe, which will also include Australia, New Zealand, Israel, and South Africa.

5.3.b Quality of Life

Appropriate statistical methods will be used to analyze QOL. Instruments to be analyzed include the KCCQ, SF-12, the EuroQoL Health Rating Scale, MOS-D

depression scale, and BSI-A anxiety scale. See Section 4.2.f for descriptions of these scales.

5.3.c Subgroup Analyses

Subgroup analyses of the primary and secondary endpoints will be performed. Subgroups will be based on baseline recordings of race (black, non-black), sex, age, presence of diabetes, ejection fraction, serum potassium, serum creatinine, use of β-blockers, use of digoxin, use of potassium supplements, first versus subsequent AMI, Killip class, reperfusion status, history of hypertension, history of HF, history of smoking, history of angina, time from index AMI to randomization, and geographic region. Subgroups based on continuous measures such as age, ejection fraction, serum potassium, and serum creatinine will be dichotomized at the median value.

5.4 Safety Analysis

All patients who receive at least one dose of study medication will be included in the analysis of safety. Routine safety data will be collected only for patients still taking study medication.

5.4.a Symptoms and Adverse Events

All adverse events will be coded and summarized by treatment group. The incidence of treatment emergent adverse events will be tabulated by treatment group and body system as well as by severity and attribution. In addition, the incidence of adverse events causing discontinuation of study medication and serious adverse events will be summarized by treatment group. Only those adverse and serious adverse events that occur within 30 days after a patient's last dose of study medication will be included in the analysis of safety.

5.4.b Vital Signs

Vital signs will be listed and summarized by scheduled time and treatment. Changes from baseline in heart rate and BP will be analyzed by analysis of covariance, with baseline value as a covariate.

5.4.c Clinical Laboratory Tests

Clinical laboratory data will be summarized and treatment groups will be compared. Within treatment group changes from baseline to post-treatment will be analyzed using a paired t-test. Differences between treatment groups will be evaluated using analysis of covariance with baseline value as a covariate. Shift tables will be used to graphically depict the shift in laboratory values. These shift tables will capture those laboratory values that are clinically relevantly high or low at either baseline or post-treatment. The incidence of clinically relevant laboratory results will be tabulated by treatment group.

5.5 Committees

In conjunction with the Sponsor, the Steering Committee, comprised of the lead Investigators from each participating country or region, will oversee the trial. There will also be an independent Data Safety Monitoring Board. All endpoints will be adjudicated by a Critical Events Committee.

5.5.a Steering Committee

The Steering Committee will be comprised of the lead Investigators of each participating country or region, the Sponsor, and an independent statistician. It will remain blinded to the trial results through the conduct of the trial. It will oversee the conduct and reporting of the trial, including developing the network of Investigators, assuring expert clinical guidance and a high standard of scientific quality and making any necessary modifications to the protocol. The Steering Committee Charter will define the responsibilities of the committee.

5.5.b Critical Events Committee (CEC)

The purpose of the independent Critical Events Committee (CEC) is to classify the cause of all endpoint events for each patient during this trial. This committee will review documentation from each suspected endpoint event (see Section 2.0), and will remain blinded to treatment assignments. The CEC will assess the nature of each event and determine whether the pre-specified criteria for the endpoints were met. The CEC Charter will define the responsibilities of the committee.

5.5.c Data Safety Monitoring Board (DSMB) and Interim Analyses

An independent DSMB will be impaneled to monitor the safety and efficacy of the trial and to determine whether sufficient treatment differences exist to terminate the trial prematurely. The DSMB will consist of five members: four cardiologists, expert in the diagnosis of heart failure and its progression; and one medical statistician, expert in the analysis of clinical trial data. One member will serve as chairperson. No members of the DSMB will act as Investigators for the study. The DSMB Charter will define the responsibilities of the committee.

Decisions on early study closure for efficacy will guided by an O'Brien-Fleming-type boundary with a Lan-DeMets alpha spending function. Recommendations of the DSMB will be reported to the Chairman of the Steering Committee responsible for enacting the discontinuation.

To maintain the blind at Searle and among other study participants, an external statistical group will prepare all aspects of the interim reports and communicate directly with the DSMB.

5.6 IVRS Center

An IVRS center will be used for treatment assignment and tracking of recruitment, withdrawal and medication supplies (refer to Section 3 for details).